Proceedings of the 34th Annual Aquatic Toxicity Workshop: September 30 to October 3, 2007, Halifax, Nova Scotia

Compes rendus du 34ième atelier annuel sur la toxicité aquatique: du 30 Septembre au 3 Octobre 2007, Halifax, Nouvelle-Écosse

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Preface/Preface

The 34th Annual Aquatic Toxicity Workshop was held at the Westin Nova Scotian, Halifax, Nova Scotia September 30 to October 3, 2007. The Workshop included two plenary presentations, 156 platform and 89 poster papers. Total attendance was 397.

This workshop was one of a continuing series of annual workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These workshops are administered by a Board of Directors, and organized by local organizing committees. The Proceedings are published with the support of the Department of Fisheries and Oceans.

L' 34ième atelier annuel sur la toxicité a eu lieu au Westin Nova Scotian à Halifax, Nouvelle-Écosse, du 30 Septembre au 3 October 2007. Le atelier a donné lieu a 2 communication lors de séances plénières, 156 exposés d'invités d'honneur et 89 communications par affichage. 397 personnes ont assisté au atelier.

Le atelier a premis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces atelier annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publies l'aide du ministre des Pêches et Océans.

Editors comments/Remarques des editeurs

This volume contains papers, abstracts or extended abstracts of all presentations at the workshop. And author index is also included. The papers and abstract were subject to limited review by the editors but were not subjected to full formal or external review. In most cases the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned nor rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The editors would like to thank Dr. Jill Graham and Ms. Monica Lyons for their assistance in the preparation of these proceedings.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longeur, ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs sont aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La lonueur et la forme des communications varient parce que ces derniéres sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur le travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialiesés ne constitue ni une approbation, ni une recommandation d'emploi.

Les rédacteurs en chef voudraient remercier Dr. Jill Graham et Mme Monica Lyons dans la préparation de ces compe-rendus.

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Plenary

Navigating new waters in aquatic toxicology: The challenges of assessing and protecting the health of aquatic ecosystems in Canada.

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Abstract

Despite decades of work and significant progress in many areas of water science and environmental protection, many stressors continue to impinge upon water bodies in Canada, from the high alpine lakes to the Great Lakes, to coastal and offshore waters of our three bordering oceans. Water quantity and quality are threatened in many places. New pervasive chemicals are found almost monthly in the environment, posing unknown risks to both ecosystem and human health. Old concerns such as sewage take on new profiles with the discovery of many endocrine disrupting substances (EDCs) and effects such as feminized male fish. Whole regions such as the Arctic, north-central Alberta, and coastal embayments are under siege by climate change, tar sands development, and unmanaged aquaculture, respectively. Recent assessments of aquatic ecosystem health in North America point to many unresolved or partially resolved issues. Never before have there been so many new challenges and needs in aquatic toxicology and related disciplines (e.g., fish toxicology, ecosystem health, risk assessment, ocean and human health, predictive modeling).

This paper offers a personal view of the challenges. In an era of profound climate change, it is imperative to reflect on our goals and plot a new course, striving for solutions to the most important problems. Above all, a sense of urgency is needed amongst practitioners to ensure that research and monitoring initiatives across all sectors (government, industry, NGOs, universities) continue to be supported and strengthened, and that new knowledge is effectively used in decision-making, policy-making, public education, and other actions to protect vital and irreplaceable aquatic ecosystems. The new challenges in aquatic toxicology are many; the courses we navigate are up to all of us in the years ahead.

1. Introduction

The 34th Aquatic Toxicity Workshop (ATW) is a threshold event: we have been meeting since 1974, now over a third of a century. This is the third ATW in Halifax,[1] and the eighth in the Atlantic Provinces. It is especially noteworthy in that 2007 is the 100th anniversary of Rachel Carson's birth. Her landmark book Silent Spring (1962) stimulated many people, the second generation of water pollution biologists and chemists, to join the

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[1] See the previous Proceedings, available on the ATW website and ATW CD.

field of aquatic toxicology (AT) and environmental science. Now many of the participants at the ATWs, either in graduate school or working in early- to mid-career, will be navigating the new waters of aquatic toxicology over the next few years, with new challenges and responsibilities galore!

The ATW community in Canada collectively evolved from what some of the key founders (the first generation) in the field in North America would call the era of "pickle jar" fish acute toxicity testing and experiments. Some of the first generation scientists were luminaries—Don Alderdice, Mike Waldichuk, Harold Harvey, Perry Anderson, John Sprague, Peter Doudoroff, Charles Warren, John Cairns, Ruth Patrick, Donald Mount, and Donald Reish are but a few of them. We all have our lists and our views of the history, all valid – see Sprague (1992, 1996) and Rand et al. (1995).[2] Due to the work of many scientists and their graduate students and associates, AT is now a recognized, structured and highly diverse discipline with many techniques across species and levels of biological organization (Rand 1995). There are now many graduate programs in ecotoxicology, a vast literature including a body of excellent reference texts, many interdisciplinary approaches and collaborations, and strong linkages to ecological risk assessment (ERA), environmental effects monitoring (EEM), environmental impact assessment (EIA), and cumulative impact assessment (CIA). There is recognition and use of AT in countless environmental laws and regulations, as well as in the management, policy, and decision-making initiatives of government and industry. Gordon Craig summarized this very well a few years ago in St. John's, Newfoundland (Craig and Wells 2000). Aquatic toxicology, with its closely linked and essential disciplines of environmental chemistry, ecological risk assessment and ecology, is clearly a vital and cornerstone environmental science.

However, despite successes of the science, many individual contributions, and the continuity of the ATW series, there are still many new challenges and needs in aquatic toxicology and related disciplines (e.g., ecosystem health, risk assessment, ocean and human health, predictive modeling). We are in an era of many profound environmental changes, led by global climate change. We are trying to protect and conserve the health and ecological integrity of aquatic ecosystems across the continent, all the while assuming that we have the necessary capacity, knowledge, public will and political will to support the effort. It is imperative to reflect on, discuss, and plot a new and energetic course in AT: how will we be "navigating the new waters" with new goals and destinations, and how will we prepare our science to meet the many challenges?[3]

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[2] Also recall the special EPA symposium at the SETAC meeting in Philadelphia, 1999, at which Don Mount and Eugene Kenaga spoke.

[3] Two caveats to these questions: One is that predicting the future is a risky business. The second is that there are only a few written histories of AT (there are some, see Rand *et al.* 1995) with which to compare the present and help soothsay the future. One of our needs

This paper covers both issues and challenges and has five key messages, grouped under the theme "Navigating New Waters in Aquatic Toxicology: identifying new goals, seeking new solutions." The messages are as follows:
REVISIT OUR GOALS: 1) Instill a sense of importance and urgency in what we are doing;[4] 2) ask and debate the big AT questions pertaining to effects of chemicals on fundamental life processes; 3) recognize and accommodate the complexity and interdisciplinary nature of the issues and science.
STRIVE FOR SOLUTIONS: 4) Continue the development of new methods, including standard methods; and 5) involve the whole community in the AT field and maintain feedback loops with decision and policy makers in environmental management.

2. Navigating new waters: An overview of some of the Canadian water (fw/sw) issues – Western, Northern and Arctic, Great Lakes and central Canada, to Atlantic Canada.
There have been decades of work, with significant progress made in many areas of aquatic science, monitoring, and environmental protection. The evidence shows that many stressors continue to impinge upon water bodies in Canada, from the high alpine lakes in the western mountains to the Great Lakes and their watersheds, and to coastal and offshore waters of all three coasts. Water quantity and quality are threatened in many places. New pervasive chemicals are found almost monthly in the environment. Old concerns such as sewage take on new profiles, with the threat of a multitude of endocrine disrupting chemicals (EDCs) having effects such as feminizing male fish. Whole regions such as the Arctic, northern Alberta, and coastal passages and embayments appear to be under siege with water quality and/or quantity issues.
Recent assessments of aquatic ecosystem health in North America, and Canada in particular, point to many unresolved or partially resolved issues. There have been many such assessments recently: Great Lakes Solec report (Scherer 2004); Pew 2003; U.S. Oceans Commission 2004; Millennium Ecosystem Assessment (MEA) 2005; Gulf of Maine Summit Report (Pesch and Wells 2004); various EC SOE reports; WWF report on the Atlantic offshore oil spills and seabirds; etc. Many water issues have been in the daily news and in the journals over the past few months, issues that involve our AT science, policy, and management capabilities. The following overview is a selection of

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is for a written history from each of the practitioners, profiling experiences, development, success and failures in AT, so as not to lose the experience of each generation of ATers. Histories could be written by each of us as we move ahead on new courses of discovery. An ATW history is also needed.

[4] We should consider drafting and releasing a proclamation or declaration at each AT workshop, emphasizing priorities, concerns, resource needs and the importance of what we are collectively achieving. As shown by the recent IPPC deliberations and releases, the time of silent science is long over.

toxic chemical and ecotoxicological issues that we know aquatic ecosystems are facing and that illustrate the diversity of stressors and the many needs for continued research, monitoring, and action.

Linkages between Human Health and Ecotoxicology

The linkages between human health and ecosystem health, including aquatic, are many. They have become well documented over the past few decades (e.g., DiGiulo and Monosson 1996). They include the infamous Minimata mercury incident in Japan, algal toxin intoxication due to domoic acid in mussels off PEI, high chemical levels in the blood and fat of native peoples across Arctic Canada, and the recent testing for persistent synthetic chemicals in blood and urine from people (including politicians) across Canada. In some cases, the linkages have been confirmed (e.g., Minimata, harmful algal blooms-domoic acid); in others, the risks are perceived but nonetheless have an impact in galvanizing action (e.g. Sydney Tar Ponds, NS). It is no longer questioned that healthy ecosystems support healthy people, and vice versa, even if the cause-effect linkages are not always crystal clear. New programs have been established, for example, on the marine side with ocean and human health institutes and programs in the USA and Bermuda. Indeed, the whole topic of "oceans and human health" was recently reviewed (Bowen et al.*et al.*, 2006).

One of our biggest challenges is in assessing the hazards and risks (these are different) to ecosystems and human health of chemicals already in use (so-called existing chemicals) and the many hundreds of newly introduced chemicals each year. I have had some experience with this challenge through work with the International Maritime Organization (IMO) and GESAMP's Evaluation of Harmful Substances (EHS) working group. The task has been to evaluate the hazards to the marine environment and human health of the many chemicals and other substances carried by ships worldwide, for the MARPOL Convention (GESAMP 2004); assignment of rankings have often been a judgement rather than a calculation using hard data. In the news recently, many chemical issues having both human and environmental health implications have been described (e.g., lead in toys, melamine in food, bisphenols from plastics). Aquatic toxicology continues to play a major role in the work of hazard and risk assessments. The challenge is how to "fast-track" such assessments, with accuracy or high certainty, given the frequent paucity of data in the three essential areas—persistence partitioning, potency/toxicity (see McCarty and Mackay 1993)—for most of the industrial chemical and mixtures in use. New approaches are helping in this process, such as small-scale approaches (Blaise and Ferard 2005), QSARs, predictive toxicology (Helma 2005), computational toxicology (Ekins 2007), etc. However, the task of conducting credible hazard and risk assessments of little-known chemicals and industrial chemical mixtures is more than challenging: relevant data on composition and the essential areas (the three Ps) are often simply unavailable to the assessors!

Western Issues, Freshwater and Salt

Water issues in Western Canada (described herein as British Columbia to Manitoba) abound, ranging from coastal water quality to water quantity concerns on the Prairies. The rapid reduction of glaciers in the Rockies and other ranges (described at the 2006 ATW in Jasper—see Martin Sharp, ATW Proceedings 2006) and the consequent reduced river flows are of particular concern – water flowing into the prairie rivers will be less and differently timed. The risks of oil spills from ships and shipping along the BC coast, and proposed oil development near the Queen Charlotte Islands, have come to the fore by two recent accidents, as has the impact of oil spills in FW bodies by last year's spill in Lake Wabamun, Alberta. The sewage issue is finally being addressed at Victoria, BC, after many years of debate and expensive ambient monitoring (Stubblefield *et al.*, 2006). Many other issues were addressed at the recent ATW in Jasper 2006 and have been previously described by Waldichuk (1988) and Schindler (2001, 2007). They include sewage/sewage management; risks and impacts oil pollution; impact of resource proposals in BC; water quantity/quality across the Prairies; chlorophenols in groundwaters and surface waters in Calgary (a 1992 risk assessment study was conducted on this); and the impacts of EDCs from various discharges (municipal, pulp and paper) in river systems. The overall effect of the tar sands development on water quantity and quality in the northern rivers is of concern too; this may be the largest western problem in the long run.

Northern and Arctic issues

Water AT issues in the North are driven by new industrial development opportunities, such as mining for uranium and diamonds, oil and gas development—e.g., the Mackenzie corridor and pipeline proposal, and the various predicted influence(s) of climate change—increased air temperatures, the melting of the permafrost, and the greatly reduced ice cover over the Arctic Ocean. This literally is an example of AT navigating new waters, as the Arctic Ocean is facing major changes as an ecosystem. Due to the reduced ice cover, there will be opportunities for year-round shipping through the previously iced-in Northwest Passage; impacts on polar bears and seals are predicted to be severe, as sea ice is a critical habitat for feeding and reproduction, respectively.

In the news in 2007, the issues have been: 1) mining development and impacts on aquatic resources in the Canadian North (Hall 2007); 2) aquatic impacts of placer gold mining, and of pit and underground mining for gold. The impacts include direct habitat destruction "as whole streams are diverted to get at the gold bearing-deposits" and "removal creates problems of acid rock drainage and recovery of concentrate often uses techniques such as cyanide leaching and complexation"; 3) Implications to marine mammals—e.g., seals and polar bears—of the greatly and increasingly reduced ice cover; 4) the opening up of the Northwest Passage to shipping, with attendant risks from spills; 5) the loss, through drying up, of freshwater lakes in the north; and 6) the build-up of persistent chemicals in food chains in the North and the effects of this on human health, particularly that of pregnant women, a problem that has not diminished despite

regulation and global conventions. As pointed out by Chapman and Riddle (2003), there are many new needs and opportunities for arctic or polar ecotoxicology—terrestrial, FW, and coastal.

Great Lakes, Central Canada, and St. Lawrence River and Estuary Issues

Water issues in the Great Lakes and its watersheds have been studied, managed, and mismanaged for decades. These have been led in part by the initiatives of the International Joint Commission and the Canada Centre for Inland Waters. The issues are well known to participants of the ATW series, as much of the AT has been conducted by central Canadian institutes, universities, and private sector since the 1970s. The Great Lakes issues were well described in 2004 at the Gulf of Maine Summit Conference in New Brunswick (Scherer 2004).

Despite many successes at pollution control and regulation, there remain many important issues: the plethora of trace toxic chemicals in the Great Lakes and its watersheds, including several that have been regulated for decades—e.g., Mirex, chlorophenols, PCBs; impacts of introduced species (Zebra mussel, sea lamprey) on other species and habitats; impact of zebra mussels on water quality; drinking water quality; impacts of legacy chemicals such as Mirex; effects of toxic chemicals on aquatic birds and small mammals of the region (M. Gilbertson, pers. comm.); and acid rain effects on lakes and watersheds in NE Ontario and Quebec. In the news recently were: 1) the continued contamination of the beluga whales in the St Lawrence estuary, 2) virus causing mass die-offs of fish in the Great Lakes (G and M, 16 May 2007)5; 3) seeking controls for lampreys in the Great Lakes (pheromones, electricity, chemicals in food) without success (CH, 22 July); and 4) discussion about the bulk export of water, currently not allowed (G and M, 13 Sept 2007; Canada's water on the negotiating table, G and M, 10 Sept 2007). Surely one of the biggest challenges is assessing the long-term effects on living resources and habitats of the pervasive and cumulative chemical exposures in some of the more industrialized lakes: e.g., Lake St. Clair, near Sarnia's industrial complex; Lake Erie; and downstream of the whole region, the St. Lawrence River estuary.

Atlantic Canada issues

The paper now concentrates on the water issues of Atlantic Canada, a geographic area of greater familiarity to the author! Many water quality issues have been in the news lately. Examples are:

Pesticides

1. PEI - pesticides and nutrients (fertilizers). Fish kills in PEI, perhaps due to pesticides from agricultural lands (CH, 16 Aug. 2007);

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[5] G and M – Globe and Mail; CH – Chronicle Herald, Halifax; TJ – Telegraph Journal, Saint John, NB.

2. Fate and effects of chemicals (e.g. herbicides such as Vision) used over blueberries; movement of residual chemicals into groundwater and well waters;
3. In the Minas Basin, Bay of Fundy, forums, 2002-03, freshwater quality was the No. 1 public issue around the Basin, due to concerns about quality of drinking water: e.g., presence of pesticide residues in groundwater;
4. Use of herbicide Vision (glyphosate as active ingredient) over woodlots in Cumberland Co., NS;
5. Agent Orange use in NB DND property at Camp Gagetown.

Acid Rain

6. Continued acid rain and Hg impacts e.g. Kejimkujik National Park (Clair, T., pers. comm.); also acid rain and acid fog impacts in Fundy National Park.

Drinking water quality

7. Linkages between groundwater and surface waters e.g. cattle feedlot nutrient and microbial contamination of wells in the upper Annapolis Valley;
8. Water quality in watersheds providing drinking water (Pomeroy *et al.*, Environment Canada, pers.comm.).

Many of the issues have been with us for years, with little resolution: e.g., acid rain impacts in southwest NS, as shown by studies at Kejimkujik National Park, and from acidic fog at Fundy National Park; pesticide and nutrient runoff and fish kills in PEI; chemicals in groundwater in the Annapolis Valley and Minas Basin watersheds; PAHs in the Sydney Harbour fish and lobsters; cadmium in the nearshore environment off Belledune, NB, lead smelter. Disturbing new issues have surfaced: e.g., the use in the 1950s of Agent Orange at Camp Gagetown, NB.

Coastal and marine issues are diverse and often reflect the influence of land-based activities on coastal resources (e.g., Wells and Rolston 1991; Environment Canada 1994; Pesch and Wells 2004; Janowicz and Tremblay 2006). They range from:

Specific contaminants

- chronic oil pollution and marine wildlife (offshore oil spills from shipping, and coastal oiling) (Wiese, F., several publications);
- effects of aquaculture sites and siting; fate and effects of heavy metals such as Hg at specific sites (Jardine 2007);
- forestry pesticides and the legacy of spraying DDT in the past;
- contaminants in sediments from dredging operations in harbours (Tay, pers.comm.);
- Hg, N, sewage as priority chemical stresses in the GOM (Pesch and Wells 2004);

Industrial and municipal operations

- the proposed White's Cove, NS, basalt quarry and marine terminal (CH, 17 June 2007) and concerns about lobsters (21 June 2007);
- potential impacts of gold mining operations (new mine proposed for Moose River, NS, and concerns about cyanide in tailings ponds, and general impacts

upon the coast of tailings discharges on the watershed and downstream waters (CBC radio, 26 Sept);

- Halifax harbour municipal effluents (now being addressed by the Harbour Solutions Project, Halifax);
- impacts of fish plant effluents on coastal water quality;
- risks from LNG tankers in outer Bay of Fundy (TJ, 14 March and11 June, 2007);
- risks associated with undersea munitions, from ships which are now rusting, or from disposed-of munitions: i.e., the sea was a dumping ground from the 1940s to 1970s, prior to the London Dumping Convention (this is also a bigger global problem, with a conference coming to Halifax in Oct. 2007);

Broader habitat issues, with ecotoxicological implications

- cumulative impacts of tidal barriers on coastal ecosystems: e.g., the Petitcodiac River (Wells 1999; Percy and Harvey 1999); upstream dams and tidal barriers and their impacts downstream;
- the dangers of ocean acidification (Doney 2006). This is considered a critical threat to organisms which build their shells from ocean trace minerals: e.g., crustaceans, mollusks, echinoderms;
- the Gully area of the Scotian Shelf – new species: effects of chemicals, especially from offshore oil and gas operations, on deep sea organisms—a completely new area of potential research.

Some of the coastal and marine issues in the Atlantic Provinces have been or are being resolved: e.g., pulp and paper mill effluents, sewage from large urban municipalities, impacts of dredging spoils, pesticides used in forestry. Some seem to be very difficult to resolve: e.g., smaller on-site sewage contamination; coastal litter/plastics (as shown by monitoring at Sable Island and by the annual coastal cleanups); offshore oiling of seabirds (Lucas and MacGregor 2006); low level chemical exposure and acid rain in southwest NS.

We also face many marine habitat issues on the Atlantic coast: e.g., benthic impacts of trawling and dredging, salt marsh conservation, contaminants in estuaries. This also points to the fact that we appear to know so little about the biology, physiology, ecology and chemical effects on species in unique habitats along the continental shelves and in the deep sea. Some issues have been in the news lately:

- Future of deep sea fish species (CH6, 5 Jan 2006).
- Protection of coral hot spots off east coast (TJ 30 July 2007, CH 11 Sept 2007).
- Species loss – new threatened species (WCU report; G and M 13 Sept 2007).

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[6] CH-Chronicle Herald, Halifax, NS; GandM – Globe and Mail, Toronto, ON.; TJ – Telegraph Journal, Saint John, NB.

- Action needed to save wild Atlantic salmon from extinction (CH 5 June 2007)
- Controversy on status of fish stocks and predicted population declines (CH 29 July 2007).
- Many articles on demise of fish stocks and loss of biodiversity on east coast.

This summary only represents an example of the aquatic issues facing the region. The issues are described by other Canadians (e.g. Pauly, Worm *et al.*, Myers – many papers).

3. Navigating new waters in aquatic/eco-toxicology: Addressing issues in the Bay of Fundy and Gulf of Maine

The Bay of Fundy and the greater Gulf of Maine are areas of the Maritimes where many of us are focusing our attention, attempting to initiate new science and interpretation that will lead to management actions of longterm value. The Bay of Fundy is known for its enormous tides, its magnificent scenery, its valued living resources, its history, its many coastal villages and towns, and the Bay's potential for tidal power; it has been settled by Europeans for 400 years, with quite marked impacts on species and habitats (see papers in Wells et al. 2004; Rolston and Wells 2006; www.bofep.org).

Knowledge about the stresses facing the Bay is crucial to finding the right solutions and sustaining the Bay for the future. The Bay is in crisis—it is rapidly being industrialized, piece by piece, incrementally, potentially affecting its habitats, biota and overall health. Developments of late being considered include the quarry at Digby Neck, the LNG terminals in Passamaquoddy Bay, the refinery expansion at Saint John, mining in the Shubenacadie River estuary, highway construction at Windsor, more aquaculture sites in the lower Bay, more coastal housing developments, etc. The long-term health of the Bay is clearly at risk or already threatened.

Every two years, a BoFEP (Bay of Fundy Ecosystem Partnership) Bay of Fundy Science Workshop is held to discuss such issues affecting the Bay and its watersheds, including progress on our research and monitoring of chemicals (single, mixtures) found in the Bay. For example, the sixth workshop in 2004 (Percy *et al.* 2005; Rolston and Wells 2006) covered "contaminants and ecosystem health" and "monitoring programs". As well, we have many meetings of the GOMC and its committees, often with forums and special talks. In 2004, we hosted a Gulf of Maine Summit Conference at St. Andrews, where an environmental overview report with a section on contaminants (chemicals and pathogens) was presented (Pesch and Wells 2004).

Collectively, this effort points out that at least four categories of chemical problems or issues confront the Bay, not unlike those confronting other coastal areas and freshwater bodies around North America and the globe. The big issues have been identified. Primary contaminant issues in the GOM and BofF are sewage (chemicals and pathogens), mercury, and nutrients (N). These remain the priorities of the GOMC. The six top GOM issues are contaminants and pathogens, land use change, fisheries and aquaculture, climate change, habitat loss, and nutrients (ESIP or Ecosystem Indicators Project 2005-06; Mills 2006). These are major issues internationally as well (Worldwatch Institute

2007. p. 100) – sewage, nutrients (N, P, others), plastics and other debris, cruise ship wastes, algal toxins, greenhouse gases, invasive species. Of course, many other toxic chemicals e.g. PBDEs, toxaphenes, organotins, etc., could be added to this list of issues. Pervasive low-level trace chemical exposures are occurring in the Atlantic coastal ecosystem and the Gulf of Maine (Wells and Rolston 1991; Harding 1992; Chase *et al.* 2001). Of particular note are PCBs, various pesticides including DDT and its residues, Hg in sediments, and possibly Cu in specific locations in the upper bay (Chou *et al.* 2003). One question, despite the huge volume of water flushing the bay twice daily, is to what degree the Bay is a reservoir for the persistent chemicals in it, cycling and recycling through local food chains. For example, E. Sunderland and F. Gobas calculate the residence time for anthropogenic Hg in Passamaquoddy Bay to be approx. 500 years (Sunderland 2005). This type of finding points to the importance of the monitoring programs such as Gulfwatch, which uses mussels to track the presence and distribution of trace chemicals around the GOM and BofF; monitoring conducted by staff at the Rivers Institute, UNBSJ, where fish and invertebrates are monitored in the Saint John River watershed and estuary (K. Munkittrick, pers. comm.); and various other programs in NB and NS coastal waters (see Ecological Monitoring and Assessment Network [EMAN] Proceedings, Hazel *et al.* 2006).

Regulatory success in the Bay of Fundy is important to track in the Bay. What has been the success of effluent-focused regulatory programs, which have been in place, in some cases, since the early 1970s (>30yrs)? Programs focused on pulp and paper, refineries, potash, battery plants, fish plants, and municipal (WTPs). Most industrial effluents at source are now non-lethal to fish in the standard 4-day trout test (IGETG, internal data). But sub-lethal toxicity can still occur if exposures are long enough (noting that "long" varies with species—30 minutes for a bacterium, 30 years for a sturgeon), as demonstrated through sensitive, biomarker-based monitoring conducted with various freshwater and estuarine fish, e.g. ecotoxicology programs such as those of K. Munkittrick, D. MacLatchy and K. Kidd at UNBSJ, Saint John, NB (pers. comm.).

There is a need to monitor a range of newly found (e.g., flame retardant compounds or PBDEs) or manufactured (e.g., silicon compounds) chemicals in the Bay of Fundy. The potential new chemical problems are not being monitored enough in the Bay and the Maritimes as a whole: e.g., PBDEs, toxaphenes, EDCs of various kinds, especially from sewage and sewage treatment plants; e.g., estrogens, personal care products, pharmaceuticals, and various chemicals (e.g., pesticides) and therapeutic agents used in salmon aquaculture operations (see recent literature and abstracts from SETAC conferences for descriptions of chemicals to be considered in current monitoring programs).

The challenges for the Bay of Fundy are substantial. As shown by the Gulfwatch program (Chase *et al.* 2001; Jones *et al.* 2007), and previous studies dating back to the 1970s (e.g., Gaskin *et al.* 1971, 1973, 1982; Gordon 1979; Zitko 1981; see reviews by Keizer *et al.* 1984 and Wells *et al.* 1997; see bibliography by Cordes *et al.* 2006), the

number of chemical contaminants which the Bay and its inhabitants are exposed to is large, with mostly unknown effects. We suspect that the ranges of concentrations of the many trace chemicals are below those causing effects in organisms in the water column, in the sediments, in the inter-tidal zones, but this is not known for certain, particularly given the number of receptors and the possible effects of mixtures. This is not a new concern; the UN-GESAMP report in 1990 expressed the UNEP concern about long-term, low-level chemical effects in the ocean (Howells *et al.* 1990). Some of the big challenges identified for the Bay, as questions, are – how can we do better/more effective/more focused research, better monitoring, and have better science-management-policy linkages on these questions? How do we tackle the unknowns and the unknown unknowns associated with chemical exposure, effects, and risks? How do we better integrate our programs, to network, to link monitoring projects and programs? How do we ensure an ecosystem approach with the "right" set of indicators and a meaningful index for the public and decision makers? What surprises are around the table—e.g., old munitions from sunken ships, given the number of chemicals in use, the modes of transport and use, and their movement through the environment when released? Can we anticipate surprises?

Numerous chemical monitoring programs operate in the Bay of Fundy and the Gulf of Maine. They range from EPA's coastal pollution assessment monitoring programs using the TRIAD sediment methods (EPA 2004) to NOAA's Status and Trends mussel watch programs (e.g., O'Connor and Lauenstein 2006), to ocean disposal chemical and biological monitoring (Tay, pers. comm., Environment Canada). As an example, the GOMC Gulfwatch mussel watch program (Chase *et al.* 2001; Taylor 2003; Jones *et al.* 2007) shows the wide distribution of EPA contaminants of concern throughout the Gulf; a North-South distribution of some of the chemicals such as PCBs, DDT, and some of the metals; and some clear signs of coastal "hotspots". In addition to Gulfwatch, there have been extensive marine mammal (seals) monitoring/epidemiology studies in the Gulf of Maine and elsewhere in the NW Atlantic (Shaw *et al.* 2005). For the Gulf of Maine, we are taking the Vital Signs approach and categorizing the indicators useful for the various major stressors (see ESIP, www.gulfofmaine.org). A fundamental understanding of the principles of ecotoxicology underlies this approach. The recent EMAN workshop and report (Hazel *et al.* 2006), activities of the land-based activities program (Janowicz and Tremblay 2006), and the Atlantic and Fundy overviews in this paper (above) illustrate a number of the Atlantic marine near-shore monitoring programs with ecotoxicological components.

4. Navigating new waters: What are some of our principal challenges? Where are we taking the field of aquatic toxicology—i.e., what are our destinations or goals?

This section presents some ideas and topics on the new waters of aquatic toxicology and where we may be heading. The topics and ideas are grouped according to five key messages; they are meant to stimulate thought and discussion, and are not intended in any way to be either comprehensive or complete. But they are also meant to make the reader

uncomfortable with the status quo of the field; in environmental affairs, this is not the time for complacency and resistance to change.
The main messages and directions for "Navigating New Waters in Aquatic Toxicology" can be grouped as follows:

Revisit our goals

1. Instill a *sense of importance and urgency* in what we are doing.
2. Ask/debate *the big questions* pertaining to effects of chemicals on fundamental life processes.
3. Recognize and accommodate *the complexity and interdisciplinary nature* of the issues and science (e.g., train our graduates for future problems).

Strive for solutions

4. Continue *the development of new methods*, including standard methods.
5. *Involve the whole community* in the AT field, and maintain feedback loops with decision and policy makers in environmental management.

Revisit our goals

4.1. Instill a sense of importance and urgency in what we are doing (in aquatic toxicology).

Identifying the big current scientific challenges in the AT field is crucial to the health and welfare of society. We should be bold in stating the challenges as such, putting societal context and true value around the research that we are conducting on aquatic ecosystems. Some of the challenges are: a) understanding interactions between ongoing climate change and water quality/quantity (see Wake *et al.* 2006, plus ATW talks on this); b) sorting out the causes of change in aquatic systems; c) applying ecosystem health, ecological integrity and resilience concepts in practical ways, useful and meaningful to the decision makers and to the public (see Walker and Salt 2006); and d) linking environmental quality to human health and well being.
A *sense of urgency* is also needed amongst practitioners to ensure that research and monitoring initiatives across all sectors continue to be supported and strengthened. Many (most?) problems need to be resolved with all haste, not shelved or mulled over for decades. Young workers should have a strong sense of purpose, importance, and responsibility.[7] New knowledge should be rapidly and effectively used in decision-making, policy making, public education, and other actions to protect vital and irreplaceable aquatic ecosystems. The pace throughout the field should be increased. Two examples illustrate the need for rapid response, involving both research and effective policies. The clock is ticking on these issues:

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[7] This was a research ploy of Jim Watson's, of DNA fame; see McElheny 2003.

The BC Mountain Pine Beetle: To quote Hartman (2007) – "the scale of potential changes (of the Mountain Pine beetle in BC) is so great that massive, integrated, long-term research-based evaluation of processes of forest change and concurrent physical limnological and biological responses is urgently needed". But will this be fast enough to make a difference? And should research delay some practical strategies being implemented right away? Clearly not, and some strategies are underway – e.g., forest being cleared and buffer zones being built.

Climate change and the Arctic: Climate change is happening very quickly in the North, with apparently an area of the Arctic Ocean equal to Lake Superior melting every year and not being replaced. We must determine what the implications are for the various chemical and other stresses that organisms will face in such changing environments, from rates of uptake and bioaccumulation of persistent "southern" chemicals through the food chains, to the fate and effects of operational oil inputs and oil spills (see GESAMP 2007), which may become more frequent if the volume of shipping increases. The field of polar aquatic toxicology and ecotoxicology, in particular, needs to be developed further (Chapman and Riddle 2003).

4.2. Ask and debate some new fundamental questions in aquatic toxicology, pertaining to effects of chemicals on life processes, species, populations, communities and whole ecosystems.

For example, each of us should ask - what are the top three new fundamental scientific questions in AT?[8] There obviously are no correct answers to this question—every answer is unique and valid. But we could, at the annual workshops, develop a consensus on some of the outstanding new questions or challenges in AT, given the status of the field known to its practitioners, and give some direction to research over the next five to ten years. We would all benefit from discussing what we know and don't know more often at the ATWs. Some examples of topics with fundamental questions follow:

Effects of chemicals on chemoreception: Many chemicals are pervasive and persistent in water and sediments, at very low concentrations, for decadal periods. What are the influences of such persistent chemicals—e.g., the major POPs (persistent organic pollutants), trace pharmaceuticals in sewage effluents, on chemoreception and chemically modulated behaviour of aquatic organisms? At what concentrations do such effects occur? How high are the risks that this is occurring in aquatic environments? Behavioural ecotoxicology is a nascent and challenging field (Olla *et al.* 1980; Dell'Omo 2002; Clotfelter *et al.* 2004; Kane *et al.* 2005; Gerhardt 2007). It is known that

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[8] The approach here may be to choose one or two and talk about them. We could also consider having a "contest" prior to, and discussion at, an ATW.

chemoreception in fish and invertebrates plays a role in food-finding, reproduction, avoiding predation, schooling, finding suitable habitats, settling and metamorphosis of larvae and postlarvae, etc. (e.g., Bronmark and Hansson 2000; Blaxter and Hallers-Tjabbes 1992; Hebel *et al.* 1997), and some chemicals are known to interfere with chemoreception (e.g., Atema's studies with lobsters and PAHs, Atema and Stein 1972, 1974) and many other behaviours (Dell'Omo 2002; Clotfelter *et al.* 2004). Given that organisms live in a complex chemical world, subtle effects of foreign chemicals on their behaviour and on the chemical receptors themselves are not unexpected, are little understood, and are or should be of great concern.

Model organisms in aquatic toxicology: How many model organisms are enough for the purposes of screening chemicals and researching and monitoring their fate and effects in aquatic ecosystems? What are our primary models for understanding the effects of chemicals on aquatic organisms? What should they be? Is the concept even valid in AT, given the enormous aquatic biodiversity? Can current genomics help ecotoxicologists narrow down the list, given the many genes that organisms have in common (see Benson and Di Giulio 2006)? There probably is not and should not be an upper limit of "model organisms" for research purposes, especially in basic research. But for screening chemicals for use and transport, for regulating final emissions, for monitoring, and for teaching/training, there are good (mostly practical) arguments for limiting the numbers and types of organisms worked with. For research, teleost fish have been used more than any other group, and certain fish are considered models—rainbow trout and other salmonids (Wells and Moyse 1981), zebrafish (Carvan *et al.* 2000, 2005; Bopp *et al.* 2006), American flagfish (*Jordanella floridae*), fathead minnows (Ankley and Villeneuve 2006), killifish/mummichogs (Weis, J, pers. comm.), sticklebacks (Hahlbeck *et al.* 2004), medaka, etc. For freshwater invertebrates, cladocerans, crayfish, and aquatic insects such as mayflies have been utilized (see Wells *et al.* 1998; G. Persoone, pers.comm.). Amphibians (e.g. frogs) are also studied. For marine invertebrates, crustaceans, polychaetes, echinoderms, and mollusks have been used (e.g. Raisuddin *et al.* 2007). In soil ecotoxicology, nematodes and earthworms are used. Environment Canada has >10 model organisms in its regulatory ecotoxicology programs (IGETG 2004). For teaching an international course in marine ecotoxicology, we have worked with Artemia larvae, urchin embryos and bacteria (Microtox) for many years. One key question is: How does one define "enough" for model organisms, given the huge biodiversity of aquatic ecosystems (e.g. 43 phyla in the oceans alone)? The linkage to questions of interspecies sensitivity to chemicals (see SETAC volume on this) is obvious. What can we learn from the human health and toxicology sector, dependent on only a few surrogates (e.g. rats, mice, rabbits, monkeys) to guide us in this discussion?

Indicators: Frequent, reliable and transparent environmental reporting (SOE) on aquatic ecosystems is needed. What are the key indicators to use for a practical measure of aquatic ecosystem health (AEH) for freshwater, estuaries, and marine ecosystems? How can these be best combined to use as an index of AEH for SOE reporting purposes? There

is a lot of effort ongoing at present. Within the ATW series, there was Jim Sherry's discussion group at the 2006 ATW (Sherry, pers.comm.). There is a major effort through the GOMC, called ESIP (Ecosystem Indicators Partnership), focused on indicators and monitoring programs for six primary issues affecting the GOM. There has been much work on the Great Lakes (H. Scherer, pers.comm.). Much has been written on the topic (e.g., see Sindermann 1996; Burger and Gochfeld 2001; Adams 2002; Jorgensen *et al.* 2005; Wells 2003, 2005; Worldwatch Institute 2007). Can we reach a consensus on the various terms – AEH, MEQ, health, MEH, ecological integrity, etc.? Can we improve on or better utilize the EPA traffic light approach (EPA 2004) to develop an index for AEH and ecological integrity, for the purposes of state of environment reporting? There are many monitoring programs, and they appear to be mostly short-term and under-funded, using many indicators without overall coordination (e.g. Hazel *et al.* 2006). A challenge for AT is to assist with the sorting, priorization, and longevity of the key monitoring programs and most useful indicators. Given the rate of global environmental change, including climate change, this challenge takes on a new urgency.

Mixtures: How do we approach the measurement of effects of mixtures, and the cumulative effects of mixture exposures, at this time (circa 2007)? There have been a lot of recent studies on mixtures (e.g., Broderius 1991; Calabrese and Baldwin 1993, Appendix 3; Yang 1998; Deneer 2000; Mothersill and Austin 2003; Lydy *et al.* 2004; Brain *et al.* 2004). Can we assume that most industrial chemicals (largely organic) of similar modes of toxic action interact additively, rather than synergistically? How do we distinguish between natural and anthropogenic factors influencing ecosystems? This distinction may be clear for some stressors, such as DDT and thin egg shells, but very difficult to establish for others: e.g., sediment dynamics and burdens in coastal waters. Note Dorward-King *et al.*, in Baird and Burton (2001): Is it true that "the assessment of chemical stressors is relatively straightforward after 30 years of study"? Much more consideration of the testing and assessment of low-level chemical mixtures should occur at the ATWs.

There are also some fundamentally important science-management/community action questions:

a) How do we maintain long-term funding for essential monitoring programs? How do we better engage communities here? (Also see Message 5.)

b) How can we collaborate with or take advantage of the international DNA bar code project (every species of organism being specifically identified) to make it work on behalf of our monitoring programs?

c) How do we efficiently stay abreast of new developments and information in this field? How do we achieve a balance of specialty and breadth at the same time, given the enormous increase in information and knowledge? We all need advanced training in informatics and information management.

Climate change may influence many aspects of pollutant exposure and biological effects, as changing temperatures and other variables affect migration, breeding, date of first

appearance, and distributional patterns or range (Worldwatch Institute 2007, p. 94). The Millennium Ecosystem Assessment (MA) report of 2005 predicted that "the impacts of climate change on biodiversity across all ecosystems will increase very rapidly" (Worldwatch Institute, 2007, p 94-95). The most recent IPPC predictions are for higher air temperatures, more droughts, more storms, sea level rise, melting of sea ice and glaciers, etc., all of which are predicted to occur in this new century at fast paces and will influence aquatic ecosystems profoundly. How organisms adapt and whether aquatic ecosystems have the required resilience to adapt are critical questions (see Pew 2002; Walker and Salt 2006). This is where aquatic toxicology returns to its roots in environmental and comparative physiology and ecology, and where fundamental AT research must be multidisciplinary and forward thinking. Chemical stressors will be interacting on biological systems and habitats in changing ecological regimes, suggesting that AT should be considering novel multi-factoral scenarios for chemical exposures (see Pew 2002).

Invasive species: One of the most pervasive and serious threats to aquatic habitats and ecosystems are invasive species. Should aquatic toxicologists be more involved with *invasive species* programs, asking questions about chemical effects in both chemically polluted and invaded (biologically changed) environments, as well as seeking further chemically-based controls? Polluted or otherwise disturbed environments are (appear to be) more susceptible to invasives: e.g., San Francisco Bay, European waters, Black Sea (e.g. GESAMP 1997; Sindermann 1996; Leppakoski *et al.* 2002). How well understood is the interplay between the presence and success of an invasive species and the level of chemical stress in an ecosystem? Can chemicals play any new roles in controlling invasives (already applied with fire ants, zebra mussels, sea lampreys, ballast water organisms)? What are the implications of a continued chemical approach (often difficult, expensive, but successful) to further invasives control? Basically, do we as aquatic toxicologists have a new role with this issue?

Micropollutants—trace elements/heavy metals, persistent organic pollutants (e.g., pesticides, PAHs, PCDDs, PCDFs, pharmaceuticals), EDCs—all in very low concentrations in groundwater and surface waters, particularly waters for drinking water (see deVerlief *et al.* 2007); and the challenge of micro-pollutants (Schwarzenbach *et al.* 2006; see Tables 1 and 2, and section on responses to complex chemical mixtures, p. 1075): "Recent research has shown that even mixtures of compounds with different modes of toxic action may cause non-negligible effects . . . . There now are systematic investigations of mixture effects of non-interacting compounds, and countries such as Switzerland are developing water-quality criteria that include additive effects for mixtures of pesticides in surface waters." The challenge for AT is to continue to conduct realistic experiments for evidence of bioaccumulation and sublethal toxicity, as is occurring with various suspected EDCs.

Recovery and resilience ecotoxicology: Investigate further the potential for recovery[9] from chemical contaminant exposures and for the resilience[10] of chemically contaminated species and geographic areas. Do we conduct enough and any focussed « recovery and resilience aquatic toxicology »? What are some good examples of the value of such research and knowledge? Our early work in a BIO laboratory was with sea urchins that survived in low concentrations of oil spill dispersants in sea water, but later succumbed in clean sea water alone—they did not recover from exposure to very low levels of surfactants, which in short experiments were recorded as harmless. There is a recent literature in this field (e.g., Sanchez *et al.* 1999; Sancho *et al.* 2003; Depledge 1998; Zhao and Newman 2006). Every effort should be made to include recovery (potential) as a variable in experiments and standard assays.

Evaluating new chemicals: New methods should be developed to speed up the evaluation of new chemicals. As noted by Wharfe (2005), "There are more than 30,000 commercially available chemicals with production volumes >1 tonne, and 5200 of these >1000 tonnes; ecotoxicity testing continues to have a central role in the risk assessment and evaluation of chemicals"; "as a result of many technical developments (e.g., biosensor technology, portable field equipment, rapid throughput toxicity tests, gene arrays and protein expression, information technology, modeling techniques, approaches to improved diagnostic capabilities), risk frameworks are continually under development to improve the level of certainty in risk based decision making" (Thompson *et al.* 2005: Environmental Toxicity Testing, Ch. 1, J. Wharfe).

Making use of new tools in ecotoxicology: As noted by DeCoen *et al.* (2005), "More quantitative relationships between the various levels of biological organization need to be established in the global implementation of the various ecotoxicological tools and methods"; "One of the main areas of uncertainty in AT at present is the extrapolation of toxicity data obtained from lab testing to effects in real-life ecosystems." There are six future needs in ecotoxicology: improved biomarkers, transgenic systems, novel markers at the proteome level, organism level effects, mechanisms of reproductive toxicology, realistic effects assessments by predicting effects of mixtures, and ecological complexity in toxicity testing—interactions between pollutant stress and food availability. A major challenge is to optimize the knowledge related to the increased volume of toxicological data to construct molecular and genetic pathways explaining the mechanism(s) of toxic

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[9] Recovery has been defined as "the process leading to partial or complete restoration of a cell, tissue, organ or organism following its damage from exposure to a harmful substance or agent" (Natl Libr Medicine USA web site).

[10] Resilience is "the amount of change a system can undergo (its capacity to absorb disturbance) and remain within the same regime—essentially retaining the same function, structure, and feedbacks (Walker and Salt 2006).

effects; increase our understanding of the consequences of life cycle exposures to chemicals and chemical mixtures; and increase genomic information for the species widely used in ecotoxicology: "in toxicogenomics, a major application of gene expression profiling is to understand the genetic variability and susceptibility to toxic stress within ecosystems" (Thompson *et al.* 2005, Environmental Toxicity Testing, Ch. 11, DeCoen *et al.*).

4.3 Recognize and accommodate the complexity and interdisciplinary nature of the issues and science (e.g., train our graduates for future problems)

Aquatic Ecosystem Health or AEH: We need to consider what we are doing in AT within the broader field of *"aquatic ecosystem health (AEH),"* (assuming that we agree with the concept of ecosystem health), and the objective of maintaining high levels of AEH and ecological integrity. Make the linkage clear: *aquatic ecosystem health (AEH) is linked to human health in many ways!* Examples: from the "ocean and human health" programs in the US and Bermuda, such as Eric Dewailly's studies on trace contaminants in Arctic peoples and linkage back to food chain accumulations in fish and mammals; from Susan Shaw's MERI research on persistent chemicals and their effects in seals; from Kelly *et al.*, a recent study on "food-web specific biomagnifications of persistent organic pollutants" (Kelly *et al.* 2007).

Increase AT linkages formally to the science and practice of limnology. We need to have more formal sessions in limnology at the AT meetings and discussions. To quote Hartman (2007) on forestry and fresh water in BC: "....it is unlikely anyone can accurately project the impacts of this sequence of events starting with the extensive clear cutting indicted, followed by the beetle infestation and logging salvage impacts, and followed yet further with potential massive wildfire. The nature and scale of primary effects on lake and river limnology, hydrology, sediment loading, lake and stream temperature regimes, and water chemistry and temperature, can only be guessed at."

Advances in ecological risk assessment: there should be "the continued development of an integrated approach in risk assessment . . . to meet the many challenges ahead" (Thompson 2005).

Strive for solutions

4.4 Continue the development and refinement of new methods, including standard methods.

The use of bioassays is global and growing, particularly for regulatory and non-regulatory effluent testing (Power and Boumphrey 2004):

Environmental protection programs: "programs aimed at preserving water quality must have access to comprehensive toxicity screening tools and strategies that can be applied reliably and universally"; continue to apply the TIE/TRE approach; use the test battery approach in assessing risk; continue to conduct studies on the relationships of results of batteries of sublethal toxicity tests (fish, invertebrates, algae) and receive water

measurements of fish and benthic invertebrates; continue the progressive development of Chapman's SQT (sediment quality triad) approach, as it has evolved from measuring sediment toxicity, chemistry and community structure to those, plus measures of bioaccumulation, sediment stability, contaminant body residue and TIE analyses (Blaise and Ferard 2005).

Consider further the advancements in toxicity testing, as discussed annually by IGETG, and its gradual deployment into regulatory tests for water, sediments, soils, tissues, etc. (Use the IGEGT CD 2000 and recent IGETG reports). Highlight the contributions of IGATG/IGETG. According to Ostrander (2005), the surge of new AT techniques is enormous. How does one sort and prioritize these for further development into standard methods and protocols? Consider having a session at each AT on advancements in testing methodologies.

4.5. Involve the whole community in the AT field, and maintain feedback loops with decision and policy makers in environmental management.

Strengthen linkages between the ATW series and other groups and activities, perhaps more formally than in the past. Such groups include IGETG, EMAN, GOMC, Canadian Water Network, the Canadian Association on Water Quality, SETAC, and various specific EC units dealing with emergencies, SOE reporting, and effluent testing and monitoring. (Re)emphasize the networking value of the ATW series.

The value of syntheses aimed at decision makers: For example, the Worldwatch Institute continually produces syntheses aimed at the decision and policy makers: e.g., the hidden threat of groundwater pollution (Sampat 2000); the critical need to safeguard freshwater ecosystems (Postel 2005). A summary of highlights from each ATW could be prepared and sent to senior decision makers to alert them to needs and priorities in this field. International agreements and legislation at all levels provide an important basis and direction for the work of the ecotoxicologist, and can involve the prioritization of substances of concern, hazard assessment, risk evaluation and management, and expert opinion.

As a local example of the whole community being involved, Nova Scotia is currently developing a Water Management Strategy, addressing issues of water quantity and quality across the Province. It is a three-year project, and there will be public input (CBC radio interview with Jessica Patterson, 26 Sept. 2007). Such strategies should be discussed in roundtables at the ATWs; there should be science advice offered to such initiatives, informally.

5. Conclusions

The annual ATW workshop is an important mechanism for ensuring that we individually and collectively are working efficiently on the important and regionally significant questions and issues, and that the new information and understanding from our science is directly useful for decision makers and policy makers in the key environmental and resource agencies. We are clearly navigating new waters in AT: there are many issues

affecting the aquatic environments of Canada and indeed North America, and we have increasingly restrained fiscal resources and capacity. We should also not be reticent about making known the importance of what we are collectively doing and our collective dissatisfaction with the current uncertain funding of scientific programs in government departments critical to the protection of water resources and the conservation of wildlife and wildlife habitat in Canada. This continual confusion and uncertainty around reliable funding affects the morale of even the most dedicated people, puts crucial long-term programs into jeopardy, and discourages the next generation of aquatic scientists to seek careers in aquatic science and toxicology. Ours is a successful science but we clearly face many future challenges. The routes we chose to navigate, the obstacles we overcome, and the new discoveries we make are up to all of us in the years ahead.

Note: This paper is dedicated to the memory of a distinguished colleague, Dr. (Professor) John S. Gray (1941-2007), University of Oslo, Norway, a truly outstanding marine benthic ecologist who was dedicated to the protection of the global oceans and their biodiversity.

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Winners of Dr. Richard Playle Awards

Session Chair/ Président Lucy Lee

Spatial and seasonal patterns of mercury bioaccumulation in yellow perch from the St. Lawrence River at Cornwall, Ontario. A. Fowlie[1] (PL)

[1]Queen's University, Kingston, ON

The Cornwall river waterfront (St. Lawrence River, Ontario, Canada) has received inputs of mercury (Hg) from decades of industrial pollution, resulting in legacy sediment contamination. Fish are also contaminated, but whether sediments are the primary source is not clear. Yellow perch are both an important forage fish species and sport fish, and therefore a potential vector for Hg exposure to both top predators and humans. The first objective of this study was to describe the seasonal and spatial patterns of Hg bioaccumulation in yellow perch *(Perca flavescens)* from 2 contaminated and 2 uncontaminated depositional zones within the Cornwall Area of Concern. Surprisingly,

fish from a contaminated upstream zone contained significantly more mercury than fish from a contaminated zone only 2 km downstream. Length-standardized yellow perch from the upstream contaminated site had concentrations 2-4 times greater than yellow perch from other contaminated and reference zones. Additionally, evidence for a seasonal trend in reduced variability in the logHg-size relationships is presented. The second objective of this study was to test whether differences in the food web at each zone could account for patterns of Hg observed in yellow perch. Analysis of diet composition suggests that a common prey source with elevated Hg concentrations may be the primary route of methylmercury (MeHg) exposure. However, there was no consistent significant difference in trophic structure among zones, as indicated by stable isotope and stomach content analyses. Rather, patterns of mercury in yellow perch may be attributed to the heterogeneity of contamination in sediments and prey, seasonal and diurnal movements into and out of the contaminated zones, and fish bioenergetics. A potential model for Hg accumulation in yellow perch is presented, outlining further areas for research.

Real-time RT-PCR analysis of PPARα and acat1 expression in zebrafish: early development profile and fibrate regulation. H. Parnas[1] (PL)

[1]*University of Ottawa, Ottawa, ON*

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear receptors that regulate peroxisomal β-oxidation of fatty acids and other aspects of lipid homeostasis. Fibrate drugs that target human PPARα are prescribed to treat hyperlipidemia. The fibrates gemfibrozil (GEM) and bezafibrate (BEZ) have been detected in Canadian wastewater effluents, but their effects on fish are not well studied. Primers were designed for PPARα, acetyl-CoA acetyltransferase 1 (acat1), β-actin and ef1α genes in zebrafish (Danio rerio). Following optimization of RNA isolation methods, total RNA was isolated from pools of whole embryos at 1, 6, 12, 24 and 72 hours post fertilization (hpf), and from adult brain and liver tissues. Real-time RT-PCR was used to measure relative transcript levels of PPARα, acat1 and the housekeeping gene β-actin during development and in adult tissues. All genes were detectable at all developmental stages. Transcript levels of PPARα, acat1 and β-actin increased approximately 67-, 25- and 7-fold from 12 to 72 hpf. Levels of all genes were similar between sexes in both brain and liver of adult zebrafish. Four day old larvae were exposed to 5 μM waterborne BEZ or GEM in 0.01% ethanol, and changes in transcript levels relative to the ethanol control treatment group were determined by real-time RT- PCR and normalized to ef1α. BEZ significantly lowered acat1 levels by 1.4-fold and lowered PPARα 1.5-fold ($p=0.152$). With further refinement, this model could become useful in screening chemicals and environmental samples for PPAR activity.

Biological Test Methods Development and their Application

Session Co-chairs/Présidents: Rick Scroggins and Lisa Taylor

Trends in Method Development: is the LC50 test the dinosaur of toxicology? L. Taylor[1], L. Van der Vliet[1] and R. Scroggins[1] (PL)

[1]Environment Canada,Ottawa, ON

The Method Development and Applications Section of EC's Biological Methods Division published its first set of acute lethality test methods using the single concentration or LC50 endpoint in 1990. At that time, the demand was for aquatic toxicity tests which used well-defined, easy-to-measure endpoints. Over the past 17 years, there have been an additional 18 biological test methods published that measure more subtle responses and utilize different representative organisms that inhabit environmental compartments other than water (e.g., surface soil, pore water, whole sediment). The key forces driving the development of alternate methods during the last two decades have been: 1) reduction of required resources (i.e., organisms, economics, space and time); 2) new scientific knowledge (e.g., new endpoints that reflect a better understanding of mode of action at a cellular level); 3) a demand for effects endpoints that are more relevant in risk assessment and long-term environmental management; 4) an understanding of factors which modify toxicity; and 5) recognition of the fallacy of the "one test organism fits all" approach for diverse ecosystems (e.g., the need for a test battery, regionally-relevant species, assessment of non-temperate environments). Innovation in our scientific community has given us many exciting possibilities for test methods "on the horizon." The development of "omics" technologies has been a major breakthrough in the last decade, one that has touched many disciplines, including toxicity method development. Research using genomic techniques may eventually provide predictive or early warning tools indicating contaminant toxicity before the more classical toxicological endpoints are observed. Other nascent areas of test method research include on-line biomonitoring (biosensors), in vitro tests (tissue culture), and micro-scale tests. Given the future landscape of environmental toxicology, will the LC50 test become a relic of the past?

Assessment of methods for Cnidarian early life stage toxicity tests and immunotoxicology analyses with a model hydrozoan, <u>Hydractinia echinata</u>. M. Nipper[1], R. Carr[2], J. T. Lisle[2], K. Strychar[1], J. Moore[3] and M. Grohmann[3] (PL)

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Pollution, disease and climate change have been implicated in the worldwide decline of coral reefs, warranting the need for research on coral recruitment, survival and sensitivity to disease. Slow coral growth, infrequent spawning and difficulty of laboratory maintenance are strong impediments for research with coral species (Cnidaria,

Anthozoa). Polyps of the Classes Anthozoa and Hydrozoa exhibit similar morphological structure, with radial symmetry, a mouth encircled by tentacles, and a gastrovascular cavity. The embryo endoderm produces the gastrodermis, the ectoderm produces the epidermis and a net of nervous cells, and there is a non-cellular mesoglea between the endo- and ectoderm. The homologous nature of the body structure and systems of these two Cnidarian classes suggests that colonial hydroids would be good surrogates for reef forming hermatypic corals. Therefore, a model hydrozoan, Hydractinia echinata (Hydrozoa, Hydractiniidae) has been selected for early-life stage toxicity tests analyzing the effects of contaminants on larval development and metamorphosis, and for the assessment of the effects of contaminants on immune ability. Toxicity tests with several categories of contaminants, including organochlorine pesticides, polycyclic aromatic hydrocarbons and metals, were performed for the assessment of larval development and metamorphosis. Effects of contaminants on the immune ability of adult colonies will be assessed by analysis of microbial activity inhibition, and the presence and frequency of apoptotic and necrotic cells, commonly used as immune biomarkers. The potential applications of these methods for coral reef management will be discussed.

Environment Canada's echinoid fertilization test: The need for change. J. Miller[1], C. Buday[2], K. Doe[3], P. Jackman[3], D. McLeay[4] and R. Scroggins[2] (PL)

[1]Miller Environmental Sciences Inc., King City, ON; [2]Environment Canada, Ottawa ON; [3]Environment Canada, Moncton, NB; [4]McLeay Environmental Ltd., Vancouver, BC

Under the Disposal at Sea Program, Environment Canada's (EC's) "Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars)" uses echinoid fertilization in sediment porewater to help evaluate the suitability of dredged material for ocean disposal. Under the Environmental Effects Monitoring Program, the toxicity of pulp and paper and mining effluents discharged to the marine environment is assessed using this test. In recent studies on highly contaminated sediments from Sydney Harbour, NS, the echinoid fertilization test gave results inconsistent with the expected contaminant gradient and the results produced using other sediment toxicity tests. Research initiatives, undertaken to improve the sensitivity of the echinoid test, and to minimize background ammonia toxicity as a confounding factor, led to the investigation of a new echinoid embryo/larval sediment-contact test. It was later recommended that this method be developed, validated, and standardized as a new reference method for the Disposal at Sea Program. For monitoring the quality of industrial effluents, however, EC's echinoid fertilization test has been very successful. Since last amended in1997, laboratories conducting the fertilization test have made numerous suggestions to improve the method and EC has decided to proceed with the preparation and publication of a second edition. The scope of these changes will be discussed.

Summary of method development research results for the echinoid embryo/larval sediment-contact test. C. Buday[1], K. Doe[2], P. Jackman[2], R. Scroggins[1] and G. Van Aggelen[3] (PL)

[1]Environment Canada, Ottawa, ON; [2]Environment Canada, Moncton, NB; [3] Environment Canada, Vancouver, BC

Method development research has been conducted during 2005/06 and 2006/07 by Environment Canada's Pacific and Atlantic Laboratories for Environmental Testing, using an echinoid embryo/larval sediment-contact test at low sediment to water ratios with four test echinoid species: Lytechinus pictus, Arbacia punctulata, Stronglyocentrotus purpuratus and Dendraster excentricus. Embryo development tests were conducted with uncontaminated reference sediments from the east coast of Nova Scotia (porewater and sediment-contact tests) and compared to fertilization tests on porewater. The sediment-contact test has potential to minimize background ammonia toxicity as a confounding factor. Contaminated sediments from a gradient of sediment contamination in Sydney Harbour, Nova Scotia, and reference and contaminated sediments from the west coast of British Columbia have shown good sensitivity of the test species using the sediment-contact test. A dilution series of PAH contaminated sediment (NRC Certified Reference Material HS-3) and uncontaminated reference sediment (Roberts Bank) produced a good dose response using the sediment-contact test. With many of the sediment samples, the echinoid embryo/larval sediment-contact test results corresponded to the chemical analysis results. The test appears promising for use in Canada's Disposal at Sea Program. A summary and discussion of the test method and research results will be presented.

Assessment of contaminant bioavailability and environmental quality of soils using a multi-marker approach. P. Robidoux[1], V. Bérubé[1] and G. Sunahara[1] (PL)

[1]National Research Council Canada, Montreal, QC;

Simultaneous contamination of soil by various substances (contaminant mixtures) presents a challenge for risk assessment. Chemistry analyses give the level and type of contaminants. Toxicity tests assess the effects of bioavailable compounds to selected species using standard important endpoints (survival, growth, fertility). However, conventional toxicity tests give limited toxicological information and do not consider variability (e.g., temperature, humidity) of current and future field conditions. Improvement of standard assays by adding alternative biological endpoints (cellular, biochemical, molecular biomarkers) can help to understand the toxicity observed and give the appropriate information for the selection of biological parameters for other tier assessment levels (e.g., field mesocosm assays, field studies) where standard chronic endpoints cannot be used. Use of selected biomarkers alone gives appropriate information on chemical stress, range and class of contaminants, and health status. This paper presents case studies, advantages and limitations of an integrated approach including earthworm toxicity tests and a suite of biomarkers and chemical analyses. Parameters

such as lysosomal membrane fragility of cœlomocytes (neutral red retention time) can be used to assess the chemical stress whereas the antioxidant system (catalase and superoxide dismutase activity) and detoxification metabolism (glutathione S-transferase activity, metallothionein), as well as the immune activity and the contaminant uptake are used to assess the bioavailability of contaminants and the health status of the exposed organisms.

Biological testing of soil using boreal forest plants. M. Moody[1], J. Princz[2] and R. Scroggins[2] (PL)

[1]Saskatchewan Research Council, Saskatoon,SK; [2]Environment Canada, Ottawa, ON

In 2003, the need for toxicity tests using boreal forest species was recognized as a priority for Environment Canada; in response to this initiative, the Saskatchewan Research Council has been developing standardized toxicity tests using six boreal forest plant species. Initial test method development involved the selection of ecologically-relevant plant species, an assessment of plant growth in a variety of reference forest soils and towards to boric acid, metal and petroleum hydrocarbon (PHC) contaminated forest soils. Further development has included an investigation of the pH tolerance of the test species, and assessment of field-collected weathered PHC and brine-impacted soils. The soils were collected in impacted areas in northern Alberta. Intact soil cores were collected to provide a comparison in plant growth relative to traditional soil dilution tests using bulk-collected soils. To assess the performance of three test species in a control or reference soil, intact soil cores were also collected from two reference locations in Saskatchewan. The pH tolerance of the six boreal plant species was investigated at pH 4 to 7 in a standard formulated artificial soil.

At least two seed sources from across Canada for each of the plant species were also investigated to identify significant variations in germination, emergence and growth that could potentially affect test measurements and data interpretation. Two seed sources that performed similarly were obtained for five of the six species.

New territory: Toxicity test methods to assess contaminated boreal forest soil. L. Van der Vliet[1], J. Princz[1], M. Moody[2] and R. Scroggins[1] (PL)

[1]Environment Canada, Ottawa, ON; [2]Saskatchewan Research Council, Saskatoon, SK

Boreal forested and northern regions account for over 50% of Canada's land mass, yet there are currently no standardized soil toxicity test methods suitable for the assessment of contaminants in forest soils. This has been recognized as a significant data gap, given the number of resource-related industries operating within these regions (e.g., petroleum, mining and forestry). To address this need, Environment Canada, together with the Saskatchewan Research Council, has been working towards the development of a suite of applicable terrestrial toxicity test methods. Our approach to the development of boreal forest-specific test methods focuses on two aspects: (I) use of ecologically-relevant species, and (ii) modification of the soil testing system to mimic the boreal forest

environment. The groups of organisms that we are currently working with are boreal forest plants, mites, springtails and earthworms. Most invertebrate species were field-collected at different sites across Canada. We are researching the optimum culturing conditions for these species in the lab, but in some cases, sustaining populations of these organisms in the lab may not be feasible. Where lab cultures have been established, preliminary testing with field-collected site soil has begun. Unlike agricultural (tilled) soil, the distinct and separate layers of forest soil are vital to understanding and differentiating the biological activity in this ecosystem. A key component of the site soil collection was maintaining the different layers of forest soil through collection by horizon. Individual layers were separately mixed in the lab, and then re-assembled inside the test units in proportions that mimic those found in the field. To date, site soils tested include reference ("clean") forest soils collected in Saskatchewan, reference soils collected in Alberta, and hydrocarbon- and salt-impacted soils collected in Alberta. For invertebrates, the biological endpoints investigated have focused on reproduction and adult survival. The data collected from preliminary experiments can be used to determine: (I) "baseline" reproduction in uncontaminated soil, (ii) if these new test organisms are sensitive to the contaminants present, and (iii) how their sensitivity compares with standard test species.

Biomonitoring the safety of aquatic environments with fish cell lines. ***L. Lee[1], K. Schirmer[2] and N. C. Bols[3] (PL)***

[1]Wilfrid Laurier University, Waterloo, ON; [2]UFZ Centre for Environmental Research, Leipzig-Halle, Germany; [3]University of Waterloo, Waterloo, ON

Monitoring of water sources for toxicants or potential toxicants is of high priority, and although analytical instruments are available that can detect traces of various contaminants, no machine can actually determine whether compounds are toxic or not to living organisms. Regulatory testing of industrial effluents often requires fish lethality assays which can be laborious and costly. Thus, alternative evaluation methods that are simpler and cheaper but still sensitive and relevant have long been sought for the testing of effluents or of individual chemicals. This is especially relevant when new synthetic chemicals are discharged annually to water bodies around the world. We present here, over twenty years of research with fish cell lines as possible alternatives to animal testing and as useful biomonitors of aquatic environments. The maintenance and handling of fish cell lines is simpler than for mammalian cell lines, an aquatic animal facility is not needed and quantitative measures of toxicity can be obtained rapidly, reproducibly and effectively. Sample applications with selected chemicals, industrial effluents, oil sands process-affected waters, and more recently with nanoparticles will be presented. Advantages over the use of mammalian cell lines, such as the ability to directly expose cells to effluents without the need of extracting chemicals will be discussed, as well as presenting our recent work with newly developed fish cell lines that express origin-specific, differentiated functions that can be useful for elucidating mechanisms of action of toxicants.

Toxicity at the nanoscale level in trout hepatocytes exposed to quantum dots- exploring a new realm of toxicity. F. Gagné[1], D. Maysinger[2] and C. André[1] (PL)

[1]Environment Canada, Montreal, QC; [2] McGill University, Montreal, QC

The advent of macromolecules at the nanoscale (1-100 nm) has brought about concerns on their fate and possible toxicological effects in aquatic ecosystems. Given the size range of these colloids, they should produce effects at the same scale, which is beyond the range of smaller molecules usually considered in environmental studies. The purpose of this study was to examine the sublethal and lethal toxicity at the cellular and colloidal levels of cadmium telluride dots in primary cultures of fish hepatocytes. Freshly prepared rainbow trout hepatocytes were exposed to increasing concentrations of variously aged and coated cadmium-tellurium quantum dots for 48 h at 15oC. After the exposure period, the cells were washed in saline media and analyzed for cell viability (carboxyfluorescein diacetate), lipid peroxidation, DNA damage, metallothioneins, labile zinc and molecular chaperones (sensitive to protein folding). The results revealed that quantum dots were always toxic to trout hepatocytes but at different intensities. Although these compounds reduced lipid peroxidation in most cases, they increased DNA strand breaks at concentrations below 400 ng/mL. The levels of metallothioneins were also induced at low concentrations but were not correlated with labile zinc levels. The strongest response was observed with molecular chaperone of the 72 kDa family reaching 7 fold induction in respect to unexposed cells. The data suggest that these colloids are cytotoxic with evidence of DNA strand breaks, metallothionein induction and alteration in protein folding. The reported changes for molecular chaperone and metallothionein expression with the absence of zinc mobilization suggest a possible toxic interaction at the colloidal level. We conclude that nanotechnology have the potential to produce colloidal interactions that underlie cytotoxic effects.

Increasing the efficiency of fathead minnow reproduction: the effect of relative size on breeding potential. M. Pollock[1], F. Shelly[1], C. Rickwood[2], A. Squires[1], D. Chivers[1] and M. Dubé[1] (PL)

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Numerous factors affect the ability or choice of fishes to breed. For example, multiple studies demonstrate that the appropriate amount of light, temperature and food must be present before many species will breed in the laboratory. For many species we are also aware of social factors that affect breeding such as the size or colour of one's potential mates. Although studies on mate choice and factors affecting breeding are extensive, there remain severe gaps in our knowledge with regard to scientifically important species. For example, the fathead minnow (Pimephales promelas), used by numerous researchers around the world as a test subject in reproductive toxicology, has well established physical parameters needed to facilitate breeding but little data on preferred mate choice. For many species of fishes we know that mate choice is at least as

important as the physical parameters with regard to ensuring mating success. However, until now, we have no such data for the fathead minnow. The purpose of the current study was to examine some of the factors affecting mate choice in the fathead minnow. Results indicate that the relative size of males to females plays a significant role in whether or not breeding will occur. Specifically, we found a consistent relationship between male and female size which can be used to predict the probability of a couples breeding potential. The findings of this study could have a substantial impact on ensuring breeding success, thus decreasing the need for long pre-exposure periods and excess breeding pairs in experiments, which will decrease the cost and increase the efficiency of future studies.

Effects of the PAH retene on rainbow trout (Oncorhynchus mykiss) immune response.

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Polycyclic aromatic hydrocarbons (PAHs) are considered to be potent immune suppressors. Retene (7-isopropyl-1-methylphenanthrene) is a naturally formed alkyl-PAH that occurs associated with pulp mill effluents and petrochemicals. In this study we examined whether retene altered the immune response in adult rainbow trout *(Oncorhynchus mykiss)*. Preliminary work determined that a coconut oil/ethyl cellulose intraperitoneal slow-release implant system was an effective exposure method for retene; plasma levels, metabolite concentrations in bile and mixed function oxygenase activity in the liver peaked after three days, followed by a gradual decline up to 21 days. In an immune-challenge experiment, *O. mykiss* were implanted with 10, 1 and 0 $mg \cdot kg^{-1}$ doses of retene and simultaneously injected with inactivated Aeromonas salmonicida (A.s). There was no effect of either retene or A.s. injection on overall condition factor or spleen and liver somatic indices. Injection of trout with A.s was successful in activating an immune response as indicated by a significant increase in total white blood cell counts and serum antibody production. Immunohistochemistry using monoclonal antibodies specific for granulocyte and B-cells also supported the immunostimulatory effect of A.s. in spleen and head kidney tissue. Retene exposure appeared to affect distribution and abundance of these markers in unstimulated fish; however, in A.s.-injected trout this cell-mediated response was variable across tissues and retene doses. While factors such as route of exposure and sampling stress should be considered in further work, this study has established an effective challenge-based protocol for evaluating the potential adverse effects of other PAHs on fish immune function.

Do killifish from the Sydney Tar Ponds, Nova Scotia, Canada, overexpress P-glycoprotein? S. Paetzold[1] and S. Bard[1] (PL)

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We investigated the role of the multidrug resistance transporters P-glycoprotein (MDR1/ABCB1) and sister of P-glycoprotein (SPGP/ABCB11), both ATP-binding cassette (ABC) proteins, in conferring multixenobiotic resistance in a wild population of killifish (Fundulus heteroclitus) from the highly contaminated Sydney Tar Ponds, Canada. P-glycoproteins act as transmembrane export pumps that prevent cellular accumulation of a wide range of moderately hydrophobic xenobiotics including anthropogenic contaminants. The interaction of P-glycoproteins with phase I and II drug-metabolizing enzymes and other ABC xenobiotic transporters in wild populations are poorly understood. We examined a killifish population living in the estuarine Sydney Tar Ponds that have been contaminated since the early 1900s with polycyclic aromatic hydrocarbons (PAHs) from industrial activities adjacent to the site. PAHs and their phase I metabolites are potential P-glycoprotein substrates and inducers. We investigated whether P- glycoprotein expression was elevated in the liver of killifish from the Sydney Tar Ponds using quantitative reverse-transcription polymerase chain reaction. Cytochrome P450 1A (a phase I metabolizing enzyme) transcript abundance was assessed as indicator of PAH exposure. We also quantified gene expression of the complementary ABC transporters multidrug resistance-associated protein 2 (MRP2/ABCC2) and breast cancer resistance protein (BCRP/ABCG2) to understand their interaction with P-glycoproteins. MRP2 and BCRP predominantly transport phase II metabolites but also have some substrate overlap with P-glycoproteins. Gene expression of the phase II enzyme glutathione-S-transferase was also quantified since its metabolites are exported by MRP2 and BCRP. Our aim was to elucidate the cellular and molecular mechanisms that contribute to resistance to PAH contaminants.

A critical review of the Environment Canada Lemna minor Biological Test Method EPS 1/RM/37 – March 1999. D. Huebert[1] (PL)

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Aquatic macrophytes are important species in aquatic environments because they influence the structure and function of aquatic ecosystems. They can define predator-prey relationships, are important in nutrient cycling, alter the physical environment, and can determine the level of phytoplankton biomass. As such, an understanding of their susceptibility to environmental contaminants should be an essential component of toxicity assessment. This has been recognized by the CCME through their inclusion of macrophytes as a key component in the establishment of Environmental Quality Guidelines. To standardize the use of macrophytes in toxicity assessment, Environment Canada developed the standard bioassay method EPS/1*/ RM37 for the determination of toxicity using the aquatic macrophyte *Lemna minor*. The purpose of our report is to critically evaluate several components of the Environment Canada standard method

against fundamental concepts and understandings of toxicity assessment and duckweed biology, with particular reference to the effect of methodology on the assessment of metal toxicity. Components of the protocol that will be examined include light requirements, endpoint, species, culture medium, and media replacement. In addition, the Environment Canada method will be compared to the recently released (March, 2006) OECD protocol.

An investigative tool: Use of the Bacteroides Bacterial Source Tracking (BST) method to identify fecal pollution sources in sediment, biota and aqueous samples (fresh, estuarine or marine). M. Linssen Sauvé[1] and H. Osachoff[1] (PL)

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Environment Canada's Pacific Environmental Science Centre has adopted and expanded upon a genetic technique developed by Dr. Katharine Field (Oregon State University) that identifies the organism(s) responsible for fecal pollution in aquatic environments. Intestinal bacteria shed in fecal matter may arise from various sources, including human sewage, wild animal and bird populations, or agriculture. Traditional fecal contamination indicator techniques, like fecal coliform counts, use an organism that must be lab-cultured, make up less than 5% of an organism's intestinal microflora, and do not identify source. Lack of information makes it difficult to target remediation efforts. Field's technique uses the more prevalent (at ~30% of the gut microflora) bacterium Bacteroides, independent of the need for viability, as it requires no culturing but is based on the detection of DNA (after Polymerase Chain Reaction, PCR). Bacteroides are host-specific; therefore, detection of certain Bacteroides species identifies the culprit(s) of fecal contamination. Using this molecular technique we currently distinguish between fecal contamination caused by human, ruminant animal, pig, horse, dog and/or elk feces in samples of water (fresh/estuarine/marine), sediment or shellfish. Validation with bird primers (for gull, duck, goose and chicken) is underway, and future primer development is planned for other animals such as cat, bear and marine mammals.

The power of shared responsibility: Enabling community action to address land-based pollution of Atlantic Canada's coastal waters. M. Janowicz[1], P. Wells[2], I. Novaczek[3] and D. Tremblay[4] (PL)

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Abstract

Along the Northumberland Strait between New Brunswick, Nova Scotia and Prince Edward Island, fishers and their communities are concerned about the impact of sedimentation and water quality on benthic habitats and the valuable lobster *(Homarus americanus)* populations. Early sampling data support the perception of increased

sediment loading. These sediments, at least in part, come from watersheds where inputs include industrial farm chemicals, urban drainage, sewage and factory effluents. There is therefore a public perception that the water and sediments flowing into the Strait are contaminated with toxic chemicals that may be affecting the fishery.

Canada's National Programme of Action (NPA) focuses on land-based sources of marine pollution. The Atlantic NPA Team recognizes that responses to coastal pollution, including changes in existing regulatory frameworks, require firm evidence that particular compounds have the potential to cause harm in the environment. Before action is undertaken, regulatory agencies require monitoring and analyses contributing to an ecological risk assessment. This demands human and financial resources that are increasingly difficult to acquire.

To provide a timely response to public concerns, research techniques are needed that can quickly and inexpensively provide answers, even preliminary ones, regarding the quality of water and sediments and impacts on marine species and habitats. There are many benefits to government agencies sharing the work load with local community groups. To allow for this, sampling, analysis and quality control should be scientifically sound but not overly taxing to the human resources of the laboratories involved, the financial resources of the supporting agencies, or the capacity of the community groups.

This paper explores criteria for community-friendly coastal sampling methodologies that meet the needs of the concerned public for engagement, information and empowerment. As a concrete example, we consider the case of the Northumberland Strait and the potential roles of Aquatic Toxicity Workshop participants in assisting community groups.

1. Introduction: Ecosystem problems and research needs in the southern Gulf of St Lawrence

The degradation of marine and coastal ecosystems is generating concern globally (GESAMP. 2001 a,b, Pauley *et al.* 2002) and locally. For example, in Northumberland Strait, between New Brunswick, Nova Scotia and Prince Edward Island in the southern Gulf of St Lawrence, coastal communities are concerned about many aspects of ecosystem health, especially the impacts of sedimentation and water quality on benthic habitats and fisheries, and especially on the valuable lobster *(Homarus americanus)* populations (GTA 2006).

In recent decades, the Strait has experienced the collapse of cod, hake and other commercial species consequent to overfishing and natural and anthropogenic changes in the environment (AMEC 2007). This paper focuses on issues relating to the changing environment. Invasive species including tunicates, green crab and the seaweed Codium are causing increased costs and lost productivity in aquaculture. More recently there have been downturns in lobster, scallop, spring herring, winter flounder and mackerel fisheries, and there is clear evidence of coastal and marine habitat degradation, bacterial contamination, eutrophication and anoxia in estuaries (Somers *et al.* 1999, AMEC 2007). Fishers also report declines of smelt, silversides and eels; changes in various non-

commercial species; changes in the seasonal pattern of water temperatures; and changes in current strength (GTA 2006).

Limited water sampling in the fall of 2005 detected high loads of sediment compared to historical levels in Northumberland Strait (Ollerhead 2005). This was consistent with fishers' evidence of increased fouling of fishing gears and the smothering of formerly productive hard bottom (GTA 2006). Subsequent sampling by DFO in 2006 identified increased sedimentation close to estuaries and after rainfall events (Bugden *et al.* 2006). The sediment load is, at least in part, coming from estuaries where inputs also include industrial farm chemicals, sewage, food and fish plant waste, and, in some cases, effluents from pulp and paper mills and other industrial activities.

Heavy loads of sediment in the Northumberland Strait may also be related to reductions in winter ice and snow cover, which expose shores and uplands to more severe winter erosion. Significant increases in coastal erosion have been measured around PEI (McCulloch *et al.* 2002), along the New Brunswick shore (Environment Canada 2006, Chouinard and Martin 2007) as well as in the St Lawrence River and Gaspé Peninsula (Fraser 2007). Tidal currents in the southern Gulf circulate in a counter-clockwise direction, meaning that sediments eroded from southern Québec, northeastern New Brunswick and western PEI could be carried into the Strait.

Public interest has been captured by the discussions of water-borne sediments and their impacts, by local experience of fish kills in rivers and estuaries, and by failing fisheries in the Northumberland Strait. The growing perception is that, in addition to problems related to land use, overfishing, and climate change, toxins released into water and sediments in the Strait may have negative impacts on juvenile lobsters and other commercial species (GTA 2006). This perception is reinforced by laboratory studies in the Atlantic region (Fairchild *et al.* 2006) and evidence from elsewhere (eg. Colburn *et al.* 1995; Jorgenson *et al.* 2000; Sanderson *et al.* 2003) that indicate that some chemicals found in sewage, industrial effluents, and agricultural runoff (e.g. nonylphenols, selected pesticides, nutrients, pharmaceutical drugs) may negatively affect marine organisms. Limited sampling of benthic sediments of the Strait has been undertaken, mostly to monitor dump sites for dredged harbour materials. No severely contaminated areas have been identified, although cases of elevated heavy metals have been detected (AMEC 2007). However, as there is no regular or comprehensive sampling program in the Strait for toxic chemicals in either sediments or water, public concerns cannot be confirmed or allayed.

In the Strait and elsewhere, there is a need for long-term monitoring of aquatic ecosystems as a tool for environmental management and restoration of ecosystems. This requires committed resources over many years in order to sort out natural from anthropogenic changes and cause-effect relationships. In Canada, we are well equipped with laboratory and field staff trained to monitor and respond to problems in fresh water, as our emphasis has generally been on clean water for human consumption. Much less capacity is in place to assess and respond to problems in marine ecosystems. Now that we

better understand the sensitivity and vulnerability of marine ecosystems, the challenge is to build capacity through partnerships so that limited resources are optimally deployed, and benefits to marine ecosystems and their dependent human economies and communities are maximized.

2. Addressing the increase in public concern and needs for community engagement

People's interactions with government-led coastal and fisheries management are usually framed by policies and regulations, enforcement and penalties, rather than by consensus-building and co-operation. To become involved with management, concerned citizens need user-friendly scientific information, points of access to transparent policy-making processes, and mechanisms to influence regulatory decision-making. If these conditions are absent, people may either abandon hope or act in ways that fuel conflict. For example, confronted with the declines in Northumberland Strait fisheries and ecosystem health, concerned coastal residents have expressed anger and frustration rooted in feelings of powerlessness and cynicism (GTA 2006).

However, the growing public concern, as shown by the proliferation of watershed groups in the southern Gulf of St. Lawrence region, is a powerful potential force for positive change. To capture this potential, it is important to design or adjust both governance in general, and community based research programs in particular, to involve concerned citizens and respond to expressed community needs.

Ecological risk assessments and long-term broad-scale monitoring of the coastal ecosystem demand human and financial resources that are increasingly hard to acquire and which compete against global issues such as climate change and other issues of broad public interest such as health and education. For both political and practical reasons, therefore, government researchers and managers need to build alliances to tackle longer-term, labour-intensive monitoring, management and habitat restoration. Industries are required by law to assist in research related to their own polluting activities, but this is only part of what is needed. Coastal communities can help fill the gap if government, university or private scientists approach their work in ways that engage, empower, inform and support citizens who are willing to volunteer time and resources to the governance, protection, and rehabilitation of coastal environments.

Public engagement is an imperative that has been acknowledged in many other jurisdictions. For example, Richard Spinrad of the National Oceanic and Atmospheric Administration (NOAA) recently noted that "we must associate the real benefit of our research to the daily concerns of society. The days of expressing oceanographic research priorities in terms of physics, biology, chemistry and geology, alone, are long gone" (R. Spinrad, IMarEST Stanley Grey Lecture, Oct. 25 2007).

The benefits of engaging community groups have also been recognized by the Atlantic Team of the National Program of Action (NPA), which focuses on land-based sources of marine pollution (Janowicz and Tremblay 2006). One of the NPA's priorities, on-site sewage, has garnered the support of community groups who are seeking solutions to

bacteriological loading in the coastal zone. On clam beds, bacterial loadings above acceptable human health standards result in closures to shellfish harvesting. An NPA project that involved government, academia and community groups looked at a microbial source tracking technique that could offer answers as to the sources of bacterial loading in the clam beds.

Once a door to engagement has been opened by government agencies, a serious and long-term commitment is needed, transcending changes in political leadership and short-term government priorities. Notwithstanding the long-term implications for commitment of staff time and funding, engagement of coastal community groups in monitoring of inshore marine ecosystems is a cost-effective way to cover the territory and provide adequate and timely answers to public concerns.

A number of regional and national programs are in place through which government scientists and community groups work together, and there has been a trend of increasing community participation which can be analyzed for costs and benefits. The socio-economic returns of communities working directly to resolve the issues were highlighted in a recent report regarding ACAP (Atlantic Coastal Action Program) projects. The work done by ACAP groups, if done by the public service, would cost twelve times as much (Gardner Pinfold Consulting Economists Limited 2002).

There are also valuable spinoff benefits in terms of public education and empowerment. Active participation in research provides people with answers that are readily understood, accepted and acted upon locally. This is important because of the potential for reducing impacts on marine ecosystems through changes in people's daily consumer habits and activities.

Direct contact with marine ecosystems through engagement in a monitoring program is a powerful learning experience. It can result not only in improved understanding of marine systems but also in a deeper level of concern. Participation may also build leadership skills and capacity for planning, management, and conflict resolution in communities, which can stimulate other forms and levels of positive civic engagement.

3. Government and industry responses to community concerns: A shifting paradigm from the 1970s to the new millennium.

Since the early 1970's when environmental management became a regulatory impetus for government, coastal pollution control and fisheries management have slowly evolved into a more inclusive system that can respond to community concerns. Under the revised *Fisheries Act* (1971, 1977), regulations and guidelines governing the quality of final discharged effluents were established as a first step towards pollution control.

Toxicity was one of the criteria used to evaluate and regulate wastewater effluents, essentially applying the precautionary principle, although it was not called that at the time. At their source, effluents had to be non-lethal to rainbow trout in four-day freshwater toxicity tests. The specific effluent requirements for each industrial sector were agreed to by joint government-industry technical working groups. This approach

was uniquely Canadian (Environment Canada 1984); both the UK and USA at the time required field evidence of harm from effluents before acting on their quality. Initially, all testing was conducted in federal laboratories and soon afterwards in participating provincial facilities. There was little or no direct, applied involvement of the academic and NGO communities in the early days.

The 1980s brought the new *Canadian Environmental Protection Act* (1988), with its emphasis on toxic chemicals and dredged sediments. There was also a move to deregulation and reductions in environmental studies and support for government laboratories (Environment Canada 1984; Blaise *et al.* 1988). After the mid-1980s, effluent testing was conducted mainly by industry and consulting firms, with the government performing an audit function. With increasing involvement from provincial and private sector laboratories, the Intergovernmental Aquatic Toxicity Group (IGATG), later known as Intergovernmental Ecotoxicity Technical Group (IGETG), assisted in developing standard biological methods for toxicology and monitoring (IGETG 2004). The 1980s and 1990s also saw the strengthening of relationships between fishers and fisheries scientists, for example through the Fishermen and Scientists Research Society in Nova Scotia. Fisher-scientist collaborations provided valuable local knowledge to augment scientific understanding of species, habitats and ecosystem interactions. There were also efforts to develop community-based institutions for fisheries management (Kearney 1984). By the late 1990s, multi-stakeholder, watershed-based organizations such as ACAP (Atlantic Coastal Action Program) sites in Atlantic Canada had developed capacity for coastal water quality sampling. More recently CAMP (Community Aquatic Monitoring Program) has been developed and the EMAN (Ecological Monitoring and Assessment Network) of Environment Canada has begun to provide workshops for community groups on near-shore marine monitoring (see Hazel *et al.* 2006). University-led studies into water quality have increased in number, and training courses have been offered to non-specialists through labs such as Huntsman Marine Science Center (HMSC) in New Brunswick.

Thus, the parties involved and what they do in the water quality field has evolved from the early 1970s to the present time. Governments, previously working alone, have moved to partnerships, first with industry and more recently with community-based organizations.

That this emerging paradigm is more "community-based" is very positive. The challenges are to find and maintain funding, develop standard protocols and methods for community uses, and to engage communities in ways that take into account the complexity that characterizes most marine water quality problems.

4. Goals and parameters for community/government/industry partnerships in ecosystem monitoring

Responses to coastal pollution, including change(s) to regulations and guidelines, require firm evidence that particular compounds have the potential to cause harm in the

environment. Before action is considered, information (commonly laboratory and field monitoring and analysis) contributing to an ecological risk assessment is required by the regulatory agencies. This necessary process can be lengthy. Community efforts to assess the extent and causes of land-based pollution would increase the frequency and geographic scope of sampling. Such efforts would also build the capacity of communities to lobby for regulatory action.

As noted in the case of Northumberland Strait, gaps exist in coastal monitoring data at a time when the local economic base is threatened by anthropogenic and environmental problems (e.g. storm surge damage, collapse of fisheries, increased sedimentation in water, invasive species, toxic algal blooms). Public concern creates the opportunity and impetus for community groups to provide enthusiasm, knowledge of local issues, and skilled volunteers to the task of long-term ecosystem monitoring.

Establishing clear goals for community-based involvement is essential. Because the potential scope of community action is broad, it is important to develop a consensus among community groups, managers, and scientists on their respective roles, based on consideration of natural justice and ethics, subsidiarity, coordination needs, cost-effectiveness, and relative capacity.

One appropriate and useful community role is that of contributing to decisions on what particular types of changes are "acceptable" and in helping define what constitutes optimal "ecosystem health." These policy-level discussions help to fulfill the public's desire for a precautionary approach to pollution control and coastal and fisheries management. Involvement in the process of identifying societal choice requires a change in the way most government agencies make decisions, as community voices are currently absent from most government decision-making processes.

Communities may want to establish evidence for perceived changes in a marine ecosystem. The evidence can be used to confirm or deny the perceived changes and raise awareness and generate public pressure on decision-makers to allocate professional resources to the issues in a timely way.

Some communities may want to ascertain specific sources and consequences of pollution, so as to convince governments to strengthen specific regulations. Rigorous data collection required to influence government decisions may call for technical expertise that only professional scientists can provide. In such a case, the community role may involve other forms of civic engagement such as identifying issues, selecting sampling sites and coordinating public education. They may also identify the relevant decision-makers and engage them in the process to ensure that research leads to action.

A process of preliminary monitoring and analysis at the community level can help ensure that the scientists are focused on research questions that address community concerns. This requires communication and trust between scientists and community members. It is important that all data, whether generated by community groups or professionals, are collated and compared to other data sets by a coordinating agency, and that the results of analysis are clearly communicated back to the community.

In defining the appropriate community role, it is helpful to consider what concerned citizens as individuals or groups can do to change their own polluting activities to reduce pressure on marine ecosystems. Community partners, through programs of public education and action, can stimulate grassroots change. However, they generally require access to government funding programs that enable them to do this.

Balance is needed when allocating monitoring and research tasks. Citizens should not be shouldering a burden that properly rests with polluters or regulators. Many of the toxic chemicals in point source effluents are already regulated and industry shoulders the task of monitoring, reporting and mitigating industrial releases.

Clear understanding of the appropriate roles for all levels of government—federal , provincial, and municipal—is required. The principle of subsidiarity encourages decision-making at the closest level possible to the people who will be affected by the decision, yet at a high enough level to be properly resourced, coordinated and effective. This principle can be invoked when developing frameworks for integrated, coastal and fisheries co-management. The same principle can be applied to research tasks; they should be conducted as close as possible to affected communities but at the level appropriate to requirements for technical resources. Many types of research are beyond the technical and financial capacity of community groups and small municipalities and should be conducted by provincial or federal governments according to their respective responsibilities. These include many analytical tasks as well as regional-level data interpretation, data storage and dissemination. As taxpayers, communities have the right to expect some reasonable level of service from government agencies responsible for coastal lands and waters and fisheries.

5. Monitoring techniques for use by community groups

Community groups wanting to engage in marine environmental monitoring need appropriate and standardized sampling/monitoring methods and protocols; training; adequate equipment and supplies; quality control protocols; analysis and reporting mechanisms; and funding to hire core coordination staff. Community groups have already expressed the opinion that monitoring activities in nearshore marine environments would benefit from the types of coordination, accepted protocols and standards, and integration that have been developed for terrestrial and freshwater monitoring activities (Hazel *et al.* 2006).

Data collection methods for community use must be relatively simple but robust and scientifically defensible. It would be best if sampling and analysis could be performed quickly and inexpensively with minimal supervision. That said, there is a general recognition that there are no absolutely "simple" experimental or monitoring procedures that can be applied in marine environments; all require training, equipment and support; samples require analysis; and the acquired data must be interpreted and stored in a secure repository to allow for future use and comparison with other data sets.

A wide range of environmental monitoring techniques exist; but these need to be translated into community methods, if possible. There is work to be done to identify what groups or agencies are best suited to develop or adapt sampling methods and disseminate them to communities. Training needs are spelled out in IGETG methods manuals and other standard methods documents (e.g. APHA 1995) but capacity to provide the necessary training is still limited. Volunteers need to be trained not only to carry out sampling but also to identify and monitor the key hazard points in their own particular context (HACCP- Hazard Analysis Critical Control Points).

Community groups often focus on sampling whole organisms, documenting gross population features or recording selected water quality parameters. It would be useful to extend the tool box to include community-appropriate sampling protocols, standards, and analytical methods for various toxic chemicals in marine environments. To extend the toolbox, we need to know what types of data are required, what methods are presently available and what needs to be developed.

Methods are also in demand for measuring change at the ecosystem level (biomass, biodiversity, habitat types, and productivity). Is it possible to go further and develop methods that community groups can use to link physical changes and chemical pollutants to biological changes? Are there possibilities for communities to pinpoint sources of selected types of marine pollution?

Coordinating among various monitoring programs and projects is one of the critical elements needed to achieve community-based marine environmental monitoring. A coordinating body should:

- Connect the various groups who have knowledge and experience;
- Facilitate the sharing of information among communities, researchers and various levels of government;
- Assist communities to develop clear goals that are appropriate to their needs, capacities, and level of responsibility;
- Ensure that methods are standardized and nationally consistent and that results are easily shareable, accessible and understandable;
- Encourage communities to build partnerships with government, universities and the private sector to develop more research projects that address community needs.

6. A case for community involvement in coastal and fisheries monitoring and management: The Northumberland Strait

In the case of Northumberland Strait, coastal residents and fishers want to know what deleterious substances are present in water and sediments and in what quantities. They are interested in knowing from where such materials are being released, their fates and potential impacts.

In 2005, DFO Oceans Branch set up the Northumberland Strait Ecosystem Initiative working group (NSEI), a multi-stakeholder body involving fishers' organizations, community groups, academics and provincial and federal agencies, to assess the situation

in the Strait and make recommendations that would clarify and then address the issues of concern. The working group conducted public consultations, contracted an ecosystem overview, held discussions and negotiations, and finally issued a report with recommendations.

The public consultations showed that community people value science but also recognize the limitations and costliness of a strictly scientific approach. They favored a values-based precautionary approach to resource management so that remedial and preventative action would be initiated even if scientific proofs of explicit sources and effects of pollutants in the marine environment were incomplete. This is consistent with the precautionary approach, a key principle in the *Canada Oceans Act,* CEPA, as well as other Canadian Acts and Regulations and international environmental agreements. Precaution, as noted by aboriginal members of the NSEI working group, is also consistent with the ancestral beliefs of Eastern Canada's aboriginal Mi'kmaq people. Precautionary principles and engagement of communities are also part of an ecosystem approach – another tenet of the *Oceans Act* and other recent international agreements. Resource management objectives are ultimately a matter of societal choice, based on shared values. An ecosystem approach should therefore consider not only scientific data but also indigenous and local knowledge, innovations, and practice (Shepherd 2004).

The Strait working group recommended a shift to an integrated, ecosystem management framework that would include community participation. As noted recently by the Fisheries Resource Conservation Council (2007), inshore lobster fishers have been consistently lobbying for meaningful participation in decision-making. Environmental organizations and social scientists are similarly interested in gaining a seat at the table. The NSEI working group also supported establishing public awareness and education efforts and the introduction of locally relevant environmental education into school curricula. To meet information needs, they called for increased sampling of water and sediment quality and the mapping of changes in benthic topography and biodiversity (DFO 2007). They urged immediate action where threats were known to exist, for example to eliminate agricultural practices known to contribute to water pollution.

The costs of the necessary long-term monitoring of water and sediments in the Strait will be high but can be reduced by enabling citizen participation and costing out the value of protected ecosystem services of coastal waters. Fishing boats have already been employed in water sampling efforts (Ollerhead 2005, Bugden *et al.* 2006). In response to demands of fishers, Environment Canada has embarked on water sampling and laboratory testing of several chemicals of concern (e.g. nonylphenol and common pesticides) to determine chemical concentrations in estuaries and potential impacts on juvenile lobster (Wayne Fairchild pers comm.). There are also ACAP sites, fishers' and shellfishers' organizations and many watershed and community-based environmental groups active along the Strait. Some are already participating in CAMP; many are busy remediating wetlands, fish habitat in streams and estuarine habitats such as oyster reefs.

Existing efforts should be examined to see whether they are sufficient to address the particular issues of concern in the Strait (AMEC 2007). All of the goals and parameters identified earlier as important for effective community efforts to improve environmental quality are also necessary to move forward on the NSEI recommendations. Here, the questions are: Can methods be devised to monitor species and habitats of particular interest such as lobsters, eels, smelts and oyster beds? How can appropriate methods be transferred into the hands of community groups? Who should do the work of development and dissemination? Who should be the coordinating agency for data management, interpretation and reporting? What is the process to identify options for remediative action?

In the following section we discuss the potential for the ATW (Aquatic Toxicity Workshop) community and members of technical groups such as IGETG (Intergovernmental Ecotoxicity Technical Group) to improve, encourage and support collaborations between scientists and community groups for the purpose of coastal and marine environmental monitoring.

7. The role of ATW and IGETG in facilitating the development of community capacity

Public concern over marine ecosystem health represents an opportunity for ATW and IGETG and their many partners to revisit their role in developing aquatic toxicological methods and increase their efforts to bridge the gap between professionals and community groups involved in environmental monitoring.

The ATW community knows the field of ecotoxicology, delivers yearly workshops and short courses and consequently appears to be in a good position to take on the challenge of identifying and disseminating community methods and making connections to groups such as EMAN and the NPA, who have the potential to coordinate implementation of monitoring and reporting programs.

Participants in ATW, IGETG and associated groups right across the country could:

- Facilitate interaction with community groups
- Support partnerships involving NGOs/community groups
- Determine how to put simpler techniques in the hands of community groups, and who should be doing this development work.
- Facilitate community access to EMAN and its various networks
- Introduce specific methods and longer-term technical support to community groups
- Conduct courses directed at community groups, such as introduction to field techniques, principles of toxicology, specific physico-chemical and toxicity testing techniques
- Adapt Environment Canada—IGETG standard methods for community use
- Develop new techniques for use at the community level, and provide training
- Identify options for specific use in the Northumberland Strait (see above).

8. Summary and Conclusions

The Canadian public of 2007 exhibits an unprecedented level of environmental consciousness. A wealth of active organizations, through their efforts to improve environmental quality, fisheries management and habitats, is helping to knit together the frayed social and ecological fabric of rural and coastal communities. This situation has created an opportunity for developing a co-management system based on mutually agreed-to principles.

Governments, previously working alone, have moved towards developing partnerships, first with industry and more recently with fishers and community-based organizations. This is a positive trend for many reasons, including cost-effectiveness and contributions to public education and capacity-building at the community level. However, the effort needs to be extended more effectively into estuaries and nearshore marine environments. The challenges and opportunities of community engagement in research and management are exemplified by the case of the Northumberland Strait in the southern Gulf of St Lawrence. New governance arrangements are needed to enable collaborative work to identify problems and restore structure and function to such damaged ecosystems. Public education and empowerment is a critical requirement, as is the facilitation of the work of community-based organizations who want to help monitor, protect and restore marine ecosystems. Science needs to be seen as directly relevant to citizens whose cultures, lifestyles and livelihoods suffer the impacts of environmental degradation. Taking on this challenge conforms to the goals of the *Oceans Act,* the main principles of which include precautionary decision-making, an ecosystem approach to integrated management, and inclusion and empowerment of the public. This reflects an emerging consensus in international scientific circles on the need for holistic ecosystem frameworks for management of human activities in the coastal zone.

The many issues associated with a greater community role in governance, repair and maintenance of the ecological fabric of the coastal area require discussion between the community, the managers and the scientists. Some aspects of research, management, public education and ecosystem restoration logically require federal or provincial government support, while other tasks can be fairly shared with coastal communities. Identifying issues and devising strategies for resource management can be a shared responsibility under principles acceptable to all participants.

An inclusive and transparent process for negotiating roles and responsibilities is required, followed by development of adequate and stable support programs designed to engage, empower and inform community groups and fishers' organizations. It is important that governments do not simply download responsibility to communities without also sharing decision-making power and financial capacity. An unprecedented degree of leadership, coordination and cooperation, and dedication to long-term goals is required to ensure success.

The ATW participants and IGETG, being experts in the methodologies that are needed for monitoring marine ecosystem health, can make important contributions to this much-

needed paradigm shift. The roles they can assume are: facilitating interaction and information exchange, adapting or developing appropriate methods, providing training to community partners; and supporting further development of collaborations between government, industry, academe and community groups. We look forward to seeing the ATW participants and IGETG becoming common terms in the coastal and watershed group vocabulary.

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Lumbriculus variegates, a model for aquatic contaminants: Pharmaceuticals. J. Balon[1], N. Tytka[1] and P. Dehn[1] (PO)

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The freshwater oligochaete, *Lumbriculus variegatus*, may be a suitable bioindicator for aquatic contaminants capable of dermal absorption. Worms were exposed to four different pharmaceuticals at sublethal concentrations (Carbamazepine (CAR): 0.5 mM, Clofibrate (CLF): 0.0001 mM, Diclofenac (DCF): 0.01 mM, and Propranolol (PRP): 0.05 mM) or their respective solvent controls to monitor behavioral changes and growth.

Changes in body reversal and helical swimming were monitored on days 0, 3 and 7 post-exposure, while regeneration following mid-body ablation was measured over a seven-day period. All four of the pharmaceuticals decreased behavioral activity in comparison to the solvent controls. On day three, body reversal was reduced to 0.2% ± 0.9%, 1.8% ± 3.3%, 44.1% ± 24.6%, 47.2% ± 31.6%, activity in PRP, CAR, CLF, DCF, exposed worms, respectively; while helical swimming, the more sensitive behavior, was eliminated in worms exposed to PRP and CAR, and reduced to 46.5% ± 30.1% and 40.3% ± 31.4% activity in DCF and CLF exposed worms, respectively. Further reductions in behaviors occurred on day seven in DCF and CLF exposed worms. All four pharmaceuticals decreased regeneration in comparison to the solvent controls, but only CAR and PRP exposed worms exhibited significant decreases that resulted in additional loss of segments (-2.7 ± 3.9, - 1.2 ± 4.8, 2.8 ± 3.0, 3.9 ± 4.9 segments for CAR, PRP, DCF, CLF , respectively). Results indicate *L. variegatus* is a good bioindicator for aquatic contaminants capable of dermal absorption, and escape behaviors and regeneration may be sensitive biomarkers of exposure for pharmaceuticals.

Parental dietary effect on echinoid progeny sensitivity to toxicants. R. Nelson[1], M. Nipper[1], A. L. Lawrence, S. A. Watts[2] (PO)

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Although toxicity tests are widely accepted, method development in regards to echinoid nutrition and its relationship to toxicity is not standardized. The primary objective of this study was to determine if the parental nutrition of sea urchins affects the sensitivity to toxicants of their offspring in early life stages. *Arbacia punctulata* were fed either a fresh diet consisting of organic lettuce and carrots or a dry, formulated feed. After feeding *ad libitum* for 68 days, gametes were collected from adults on their respective diets and embryological development toxicity tests were conducted with copper (Cu), 1,3,5-trinitrobenzene (1,3,5-TNB), and sodium dodecyl sulfate (SDS) at varying concentrations for 48 hours. Embryos from parents consuming the formulated feed exposed to Cu exhibited significantly lower sensitivity than those from parents fed fresh feed, whereas the opposite happened with 1,3,5-TNB and SDS, where embryos from parents fed formulated feed exhibited significantly higher sensitivity. Effective concentrations to 50% of the organisms (EC_{50}) for formulated feed were 41.05 $\mu g \cdot L^{-1}$ for Cu, and 0.47 and 3.50 $mg \cdot L^{-1}$ for 1, 3, 5-TNB and SDS, respectively. Fresh feed EC_{50} values were 29.92 $\mu g \cdot L^{-1}$ for Cu, and 1.75 and 5.55 $mg \cdot L^{-1}$ for 1, 35-TNB and SDS, respectively. Based on the difference in the response of embryological development to different categories of toxicants between diets, parental nutrition plays a significant role in the sensitivity of the progeny to contaminants. Therefore, nutritional standardization for sea urchin diets in ecotoxicological laboratories needs to be addressed to increase the validity and reliability of the database for ecological relevance and application.

Effects of hexabromocyclododecane (HBCD) diastereoisomers on the thyroid axis of juvenile rainbow trout (Oncorhynchus mykiss). V. Palace[1], K. Wautier[1], K. Pleskach[1], M. Alaee[2], C. Marvin[2] and G. Tomy [1] (PO)

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Brominated flame retardants are widely used and increasingly detected in abiotic and biotic compartments globally. Tetrabromobisphenol, polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are most ubiquitous. HBCD is the *principal* fire retardant in polystyrene foams that are used as insulation in the building industry and for upholstering furniture. The technical HBCD mixture consists of three diastereoisomers, α, β and γ with the γ isomer most often detected. Differences in the proportions of HBCD isomers in food webs are known, but the relative potency of the diastereoisomers to induce biological effects is not known. In particular, disruption of the thyroid axis is of most concern but little is known of the diastereoisomers' specific abilities to affect the thyroid axis. In fish, the ability to convert the inactive form of thyroid hormone (T4) to the active form (T3) is mediated by T4 outer ring deiodinase (T4ORD) enzyme activity outside the thyroid glandular tissue. To address the deficiency regarding HBCD diasteroisomer specific effects on the thyroid system, effects of environmentally relevant concentrations of α, β and γ HBCD on rainbow trout T4ORD were examined using an in vitro system.

The dirt on bait lamina and other proposed end points to assess soil microbial toxicity. J. Rahn[1], L. Beaudette[1] and R. Scroggins[1] (PO)

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It is known that soil microbial health directly influences soil ecosystems. For this reason it is imperative that the soil microbial toxicity of contaminants be determined, as soil micro-organisms play a large role in nutrient cycling and organic matter breakdown. One way of determining microbial toxicity is the simple bait lamina test that assesses the feeding activity of both soil microbes and invertebrates. We used the bait lamina method to test both a moderately and a highly metal-contaminated soil, and an equivalent reference soil, collected in Sudbury, Ontario. Bait lamina test strips containing test bait composed of cellulose, bran flakes, and active coal (Terra Protecta GmbH, Berlin, Germany) were inserted into test soils and sampled over a 33-day period. The feeding activity at each sampling time was recorded as represented by the loss of bait. Results indicated that the reference soil consistently exhibited higher feeding activity than either of the contaminated soils and that feeding activity decreased with an increase in metal contamination level.

While results with the bait lamina test are promising, endemic soil microbial populations vary from one soil to another. Therefore a battery of tests is being investigated to gain an overall understanding of the microbial health in each unique soil system. The three

general areas of microbial health to be investigated are: soil microbial biomass, soil microbial activity, and soil microbial diversity and community structure. It is proposed that soil microbial biomass will be evaluated using the fumigation/extraction method. Soil microbial activity will be assessed using bait lamina, substrate induced respiration, microbial soil respiration, and nitrification testing. Soil microbial diversity and community structure will be examined using denaturing gradient gel electrophoresis, community level physiological profiling, as well as enzyme assays.

Development of quantitative histopathology tools to assess instantaneous pressure-change-induced effects in early life stages of rainbow trout (Onchorhynchus mykiss).
V. Palace[1], L. Peters[2], P. Cott[3], K. Wautier[1], R. Evans[1], B. Hanna[3] and D. Godard[2] (PO)
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Explosive-based seismic techniques to explore for oil and gas reserves beneath water bodies are a concern in northern Canada and Alaska. Industry and regulatory agencies acknowledge that the use of explosives in or near water bodies has the potential to harm fish, but gaps in our understanding remain. Specifically, identifying specific pressures that cause harm to fish is uncertain. Instantaneous pressure changes (IPC) from detonations can damage soft tissues due to expansion beyond their elastic capacities. In Canada, guidelines stipulate that peak pressure not exceed 100 kPa, but this is a LD50 value for fish species in general, and therefore is not precautionary. Alaskan IPC threshold is below 20 kPa. Damage from IPCs is not detectable without microscopic examination of fish tissues. To examine the existing IPC guidelines, early life stages of rainbow trout *(Onchorhynchus mykiss*; eggs, sac fry and juveniles) were subjected to a range of IPCs (7 to 280kPa) from explosive detonations. Histopathological effects were examined in these life stages using quantitative analyses derived for each specific tissue. Microscopic damage to craniofacial features, and soft tissues (i.e., liver, kidney, gill and swimbladder) suggests that the 100 kPA IPC guideline should be reevaluated.

Induction of EROD activity in fish fed benthic prey collected in the St. Lawrence beluga whale habitat. C. M. Couillard[1], B. Légaré, É. Pelletier[2] and M. G. Ikonomou[3] (PO)
[1]Fisheries and Oceans Canada, Mont Joli, QC; [2]Institut des Sciences de la Mer, Rimouski, QC; [3]Fisheries and Oceans Canada,Sidney, BC
EROD activity in liver and intestine was measured in fish *(Fundulus heteroclitus)* fed with polychaetes *(Nereis sp)* collected in the St. Lawrence beluga whale habitat. Nine groups of 10 adult male *Fundulus* were exposed during 21 days to nine diets composed of: 1) wild *Nereis* collected at five sites: Baie de Métis, Trois-Pistoles, Moulin à Baude, Baie Ste-Catherine and Baie Ste-Marguerite; 2) cultured *Nereis* mixed with sediments from: Bic, Saguenay, Bic spiked with Benzo[a]pyrene (SBBaP), or a highly contaminated aluminum smelter lagoon sediment (SAL). An additional group was fed with a

commercial fish diet (Nutrafin®). The diet SAL was the only diet to cause a reduction in body weight, hepatosomatic, and gonadosomatic indices. Moreover, it caused EROD induction in both liver and intestine. The different diets induced different degree and tissue pattern of induction of EROD activity. The diets Baie Ste-Marguerite and SBBaP caused induction of EROD activity in the intestine only, whereas the diets Trois-Pistoles and Baie Ste-Catherine caused induction in the liver only. The wild *Nereis* diets contained variable low concentrations of high molecular weight polycyclic aromatic hydrocarbons (HMWPAH) and dioxin-like compounds (coplanar PCBs, dioxins and furans) which are known inducers of cytochrome P4501A (CYP1A). The concentrations of HMWPAH in the diets (125 to 182 $ng \cdot g^{-1}$ dw) were correlated to EROD activity in the intestine but not in the liver. Conversely, the concentrations of dioxin-like compounds (0.85 to 4.12 $pg \cdot g^{-1}$ dw TCDD-TEQ) were correlated to EROD activity in the liver but not in the intestine. Thus, the *Nereis* collected at different sites in the St. Lawrence beluga whale habitat contain a complex mixture of bio-available chemicals which can cause induction of CYP1A in predators ingesting them and potentially other toxic effects.

What's an IGETG and is it contagious? L. Taylor[1], P. Jackman[2] and J. Bruno[3] (PO)
[1]Environment Canada, Ottawa, ON; [2]Environment Canada, Moncton, NB; [3]Environment Canada, Vancouver,BC

Over the past 30 years, ecotoxicity testing has become an integral component of environmental monitoring and compliance, environmental assessments, and setting standards. Over 20 standard test methods reflecting the contributions of researchers, data generating laboratories, regulators, and users of the data have been published by Environment Canada. Efforts to make these methods consistent and less variable across Canada have been facilitated by the actions of an ad-hoc group, the Inter-governmental Ecotoxicology Testing Group (IGETG). IGETG serves as a mechanism by which government agencies, at the federal and provincial levels, work together to promote a consistent approach to testing, with internationally recognized quality standards. The five objectives of this group are symbolized by the five petaloids of the sand dollar, one of the many test organisms represented in a standardized method. In addition to developing, validating, and publishing new toxicological test methods, the group also promotes the use of ecotoxicology testing in regulations and policies. Furthermore, the group disseminates and harmonizes new knowledge and the understanding of issues related to ecotoxicology testing, and provides scientific support to environmental conservation and protection programs. Lastly, IGETG works to establish and implement quality assurance practices in Canadian toxicology laboratories. With many projects on the go, an example of a recent accomplishment includes the development of a pH stabilization method that can be used when determining acute lethality of wastewater effluents to rainbow trout. In the future, the group is looking at standardizing a new echinoid embryo development test. This poster serves to inform those who are unaware of IGETG and its membership of the programs we support and our current activities.

Nano in nanotechnology – Does size matter? S. Chan-Remillard[1], L. Kapustka[2], and S. Goudey[1] (PO)

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You use and consume products of nanotechnology in everything from food and clothing to medical and electronic applications. Nanoparticles are defined by size—anything from 1 to 100 nm (ASTM E2456-06). The fates and effects of nanoparticles in the environment are virtually unknown. We present the results of acute toxicity tests on four classes of nanoparticles. The test substances included particles of copper, silver, silver-copper, nanoscale zerovalent iron, nanoscale zerovalent iron palladium, and zinc, and cerium oxide, aluminum oxide, barium strontium titanium oxide, carbon nanopowder, diamond nanopowder, silica nanopowder, titanium oxide, hydroxyapatite, carbon nanotube (multiwalled), graphite fibers, and fullerenes (C60). This work was done to determine if these substances posed a hazard at environmentally relevant concentrations. The freshwater tests included algal growth inhibition *(Kirchneriella subcapitata)*, and survival of the crustacean *Ceriodaphnia dubia* and fathead minnow *(Pimephales promelas)*. The marine tests included bacterial luminescence *(Vibrio fischeri)*, growth inhibition of a marine diatom *(Phaeodactylum tricornutum)*, and survival of shrimp *(Mysidopsis sp.)* and sheepshead minnow *(Cyprinodon variegates)*. The soil tests included germination, root elongation and emergence of lettuce *(Latuca sativa)*, radish *(Raphanus sativa)*, and alfalfa, worm survival *(Eisenia foetida)*, springtail survival and reproduction *(Folsomia candia)*.

Superfund sites and the development and application of ecological soil screening levels (Eco-SSLs) for chemicals of concern. S. Goudey[1], L. Kapustka[2], L. Oosterbroek[1] and A. Hawkins[3] (PO)

[1]HydroQual Laboratories, Edmonton,AB; [2]Golder Associates Ltd, Edmonton, AB; [3]Naval Facilities Engineering Service Center, California

An ecological risk assessment (EcoRA) is a structured investigative process. A list of chemicals of concern is compiled for the site. Screening level concentrations are then defined and compared to measured values or other available data. Historically, this process has been repeated for every new site under investigation because there were no standard screening level concentrations for individual chemicals. The United States Environmental Protection Agency (EPA) recognized the need and value of establishing standard ecological soil screening levels (Eco-SSLs) for Superfund Ecological Risk Assessments in 2000 (USEPA Work Group Eco-SSLs).

Eco-SSLs have been established for a number of compounds based on published data. The methodology developed for evaluating data was well documented and can be applied to other chemicals for establishing Eco-SSLs of known and comparable quality. This will be illustrated through the development of Eco-SSLs for chemicals of interest to the U.S. Department of Defense. These include metals found in bullets, signaling flares, igniters, tracers, explosives, primers, boosters, detonators, and casings (antimony, silver, barium, chromium (VI), and nickel), perchlorate and the high-melting-point explosive, cyclotetramethylenetetranitramine (HMX).

Establishing Eco-SSLs for these chemicals will eliminate the need to repeat the standard review process at every site that contains these chemicals. This eliminates the redundancy and substantially reduces the cost.

The effect of artificial sea salt preparation on <u>Strongylocentrotus purpuratus</u> fertilisation success. J. Pickard[1], C. Sullivan[1] and L. Stavroff[1] (PO)

[1]Cantest Ltd, Burnaby, BC

An echinoderm fertilisation assay is a frequently used endpoint for the evaluation of a sub-lethal effect from sediments, effluent, or chemical. The type of artificial sea salt and its preparation can produce different results when using the test organism Strongylocentrotus purpuratus. The fertilisation success of S. purpuratus was compared among treatments of different types of artificial sea salt and the preparation techniques used. Six commercial brands (Instant Ocean, Crystal Sea Marine Mix, Kent, SeaChem Reef Salt, Ocean Pure Sea Salt, and Red Sea Salt) were prepared using six different combinations: with or without aeration; in dechlorinated, hardened Vancouver city tap water or Type II deionised water; and aging for 24 or 48 hours. Overall, the greatest fertilisation success occurred in salts that were prepared with aeration in Vancouver city tap water, and allowed to age for 48 hours. Fertilisation success with sea salts prepared without aeration was generally lower than when aerated. Similar results compared to the control (filtered and sterilised Vancouver Aquarium sea water) were obtained with Ocean Pure Sea Salt using Vancouver city tap water at both aging times and DI water aged for 48 hours and Red Sea Salt in both aging times and preparation waters. Ocean Pure Sea Salt also produced similar results to the control when prepared without aeration. Crystal Sea Marine Mix produced similar results to the control when aerated, prepared in DI water, and aged for 48 hours. Fertilisation success with remaining brands and preparations was markedly lower.

Quality control and method development: Some considerations in sediment toxicity testing. J. Van Geest[1], K. Hunter[1], T. Watson-Leung[1] and P. Jackman[2] (PO)

[1]Ontario Ministry of the Environment, Guelph, ON; [2]Environment Canada, Moncton, NB

Method development and quality control considerations for biological tests are different from chemical analytical techniques. In biological tests, method development begins well before designing the actual test/exposure procedure. The starting point is the selection of an organism that will meet as many of the characteristics of an ideal test organism as possible and that is also appropriate for the purpose of the test method. Culturing procedures need to be established and evaluated in order to meet the biological requirements of that organism. Control charting of any number of culture health parameters is an effective means to track and evaluate the performance of a method. In an attempt to evaluate and harmonize both federal and provincial sediment toxicity test methods, work has been and is being done to reassess/validate several important test

parameters. Herein, we discuss a number of quality control and method development considerations in sediment toxicity testing, using the amphipod *Hyalella azteca* and the mayfly nymph *Hexagenia spp.* as model test organisms. These parameters include: an appropriate culturing/control sediment, food type and feeding rate, sediment-to-water ratios in the test exposure, and the use of acute/chronic water-only exposures for reference toxicant testing and investigative purposes. All of these parameters are incorporated into the test exposure design and have the potential to influence not only the type of quality control criteria established for the method, but also how toxicity results may be interpreted.

Evaluation of nanoparticle toxicity using fish cell lines. ***M. Bufalino[1], J. Beitz[1], B. Peitrobon[1], K. Hartlen[1], V. Kitaev[1] and L. E. Lee [1] (PO)***
[1]Wilfrid Laurier University, Waterloo, ON

The emerging and increasing use of nanoscale materials is causing growing concerns about their unintended impact on human and animal health, as well as on overall environmental health. The objective of the present study was to assess the toxicity profile of sample nanoparticles that could have uses in many industrial and biomedical applications, on fish cell lines.
Nanoparticles of various sizes, shapes and composition, and at a range of relevant concentrations were tested using trout cell lines derived from tissues that are environmentally susceptible such as gills, liver, brain, spleen, representing epithelial barrier, metabolically active organs, neural tissue and immune cells, respectively. Cellular morphology, general metabolic activity, membrane integrity and lysosomal function were assessed under control (vehicle) and exposed conditions (2 to 72 h of exposure) using phase contrast microscopy and fluorescence assays. Results of the acute exposures and their relevance to fish and the environment will be discussed.

Development of a method to detect and quantify commercial microbial products (CMPs) using quantitative polymerase chain reaction (qPCR). ***K. Bull[1], J. Hill [2], M. Douville [1] and F. Gagné[1] (PO)***
[1]Environment Canada, Montreal, QC; [2]University of Saskatchewan, Saskatoon, SK

The Canadian Environmental Protection Act 1999 (CEPA 1999) requires that animate products of biotechnology specified on the Domestic Substances List (DSL) be assessed to determine their presence and toxic potential in the environment. We are currently developing a method to detect and quantify these commercial microbial products (CMPs) using quantitative polymerase chain reaction (qPCR), in order to assess their presence in the aquatic environment. The cpn60 gene (also known as hsp60 or groEL) encodes the 60 kDachaperonin, and is a recognized phylogenetic marker. Using universal PCR primers, we have amplified and sequenced a 549-567 bp segment of the cpn60 coding region or "universal target" (UT) in 12 gram-positive bacterial strains (11 *Bacillus* strains and one *Micrococcus* strain) and in four gram-negative *(Pseudomonas)* bacterial strains specified on the DSL. A comparison of these UT sequences to reference data in cpnCB,

a chaperonin sequence database, led to the identification of signature sequence regions for each bacterial strain. Strain-specific PCR primers were designed and are currently being tested. A phylogenetic analysis permitted discrimination of all of our *Pseudomonas* strains and some of our *Bacillus* strains. The UT sequences of several closely related *Bacillus* strains were identical or nearly identical. Therefore, alternative phylogenetic markers, such as the cpn10-cpn60 or 16S-23S RNA intergenic spacer regions, will be explored for the discrimination of these *Bacillus* strains. The cpn60 gene has shown some success as a phylogenetic marker in the detection and quantification of CMPs using a molecular approach.

Oil and Gas Development and Production

Session Co-chairs/Présidents: Les Burridge, Zhengkai Li, Jan Ciborowski and D. George Dixon

Growth and biochemical responses of juvenile cod to acute and chronic exposure to production water from the Hibernia oil rig. L.E. Burridge[1], M. Lyons[1], T. Blair[1], K. Haya[1] and K. Lee[2] (PL)

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Production water was collected from the Hibernia oil production platform in June of 2006. The chemical characteristics of this water were determined and bioassays were conducted with juvenile cod. Acute responses were determined after 48 h exposure to concentrations of production water ranging from 0.06 to 5%. Seventy juvenile cod were also exposed continuously for 45 days to 0.05% Hibernia production water. At days 3, 14, 28 and 45 during the exposure, eight cod were sacrificed and liver, gill muscle and plasma were collected for biochemical analysis. In addition, length and weight data were collected and growth rates of exposed fish were compared to unexposed fish. There was no difference in growth rate in cod exposed for 45d to Hibernia production water compared to those held in untreated water under the same conditions. Analysis of tissues for MFO induction is currently underway.

Effect of produced water on cod (Gadus morhua) immune responses. D. Hamoutene[1], G. Mabrouk[1], S. Samuelson[1], H. Volkoff[2], C. Parrish[2], A. Mansour[1], A. Mathieu[3] and K. Lee[4] (PL)

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Emerging evidence from North Sea investigations indicates that produced water (PW) offshore discharge impacts the biota at greater distances from operational platforms than originally predicted. PW dispersion modeling shows that dilution by at least 240 times occurs within 50-100m, and up to 9000 times by 20 km from the discharge. We have

investigated the effect of PW on cod immunity by exposing fish to 0, 100 $mg \cdot L^{-1}$ (x10,000 dilution) or 200 $mg \cdot L^{-1}$ (x500) of PW for 76 days. At the end of the exposure, immune responses were evaluated and fish from the three groups were challenged by injection of *Aeromonas salmonicida* lipopolysaccharides (LPS). Serum cortisol levels, gill histology, as well as cell ratios and respiratory burst (RB) responses of both circulating and head-kidney white blood cells (WBCs) were investigated using cell observation and flow cytometry. Other than an irritant-induced alteration in gill cells (mucous cell hyperplasia) found in treated cod, baseline immunity and stress response were not affected by PW. LPS injection caused a significant decrease in RB of head-kidney cells and an increase in serum cortisol and protein levels in all groups. The most pronounced changes were seen in the group exposed to 200 $mg \cdot L^{-1}$. WBCs ratios were also impacted by LPS injection. Investigations are being completed to determine whether this impact was stronger in fish exposed to PW. Our results indicate an effect of PW on cod immunity after immune challenge with LPS.

Offshore Environmental Effects Monitoring programs on the Newfoundland Grand Banks: The Terra Nova and White Rose programs. E. DeBlois[1], M. Paine[2], G. Janes[3], F. Wight[4], D. Taylor[5], E. Tracy and U. Williams[3] (PL)

[1]Jacques Whitford Ltd.,St. John's NL; [2]Paine, Ledge and Associates (PLA), North Vancouver, BC; [3]Petro-Canada, St. John's NL; [4]Husky Energy Inc., St. John's NL; [5]DG Taylor Inc., St. John's NL

The Terra Nova and White Rose oil fields are located on the Grand Banks of Newfoundland, approximately 350 km southeast of St. John's. The Environmental Effects Monitoring Programs for these fields are in their fifth and third cycles, respectively, with baseline samples collected in 1997 at Terra Nova and 2000 at White Rose. Both programs rely on a gradient design for assessment of sediment quality and a control-impact design for assessment of potential effects on selected species of commercial fish. Over 45 stations are sampled for sediment chemistry, toxicity and benthic community structure at both sites. American plaice and Iceland scallop are collected in the vicinity of Terra Nova and at a control site located approximately 20 km to the southeast of the development. American plaice and snow crab are collected in the vicinity of White Rose and at four control sites located approximately 25 km to the northeast, northwest, southeast and southwest of the development. Water quality is assessed at Terra Nova and at two control sites located 20 km to the southeast and southwest. Selected results from both programs are discussed in this presentation. White Rose results are discussed in more detail in a parallel presentation at this conference.

Effects of offshore oil drilling on benthic invertebrate communities on the Grand Banks (North Atlantic). E. DeBlois[1], F. Wight[2], D. Taylor[3] and M. Paine[4] (PL)

[1]Jacques Whitford Ltd, St. John's NL; [2]Husky Energy Inc., St. John's NL; [3]DG Taylor Inc., St. John's NL; [4]Paine, Ledge and Associates (PLA), North Vancouver, BC

The White Rose (WR) offshore oil development is located on the Grand Banks, 350 km southeast of St. John's. Drilling occurs at three different drill centres rather than beneath a single fixed platform. Husky Energy conducts an extensive environmental effects monitoring (EEM) program at the WR site. The sediment component of the program includes measurement of physical and chemical characteristics, assessment of toxicity in laboratory tests, and assessment of in situ benthic invertebrate communities. Baseline (pre-drilling) sampling occurred in 2000, followed by post-drilling sampling in 2004 to 2006. The sediment is 95% sand, with polychaetes (75% of total abundance) and bivalves (15% of total abundance) dominating invertebrate communities. Regressions between community variables and distances from drill centres were compared Before versus After drilling. Concentration-response relationships between community variables and concentrations of >C10-C21 HCs (n-alkanes) were also compared among years. >C10-C21 HCs are a major constituent of the synthetic drilling muds used in the White Rose Field and an excellent "tracer" of drilling activity and drill cuttings dispersal. Effects on invertebrate communities were largely restricted to reductions in abundances of Paraonidae (Polychaeta; 10-15% of total abundance) and Amphipoda (2-3% of total abundance) near drill centres and at higher >C10-C21 HC concentrations. There was little or no evidence of drilling effects on the two most abundant taxa, Spionidae (Polychaeta) and Tellinidae (Bivalvia), standing crop, richness, diversity and evenness.

The "physical toxicity" side of oil and gas: Are seismic surveys an important risk factor for fish and shellfish? C. D. Andrews[1], J. F. Payne[1], L. L. Fancey[1], J. Hanlon[2], A. Cook[2], J. R. Christian[3] and M. L. Rise[4] (PL)

[1]Fisheries and Oceans Canada, St. John's NL ; [2]Oceans Ltd., St. John's NL ; [3]LGL Ltd., St. John's NL ; [4]Memorial University of Newfoundland, St. John's NL

In the past few years, considerable interest and controversy has arisen over the potential effects of seismic surveys performed during exploration for oil and gas deposits. Pilot studies have been carried out investigating the effects of seismic on American lobster, a commercially important species in Atlantic Canada. Exposure of lobster to low and high level seismic energy had no effects on delayed mortality, mechanosensory systems or loss of appendages. Sub-lethal effects, feeding and serum biochemistry, were observed with effects sometimes observed only weeks to months after exposure. A histochemical change was noted in the hepatopancreas of animals exposed four months previously. In preliminary studies conducted on a variety of species, including snow crab and Atlantic cod, where no "short-term" mortalities were observed, sub-lethal and possibly debilitating effects were found. Initial results indicate a need for comprehensive studies regarding the potential for seismic surveys to affect marine organisms. Microarray technologies, which may enhance understanding of seismic effects, are currently being incorporated into our studies.

Wave tank studies on chemical dispersant effectiveness as a function of wave: Implication in spatial and temporal toxicity response. Z. Li[1], K. Lee[1], B. C. Michel[2] and A. D. Venosa[3] (PL)

[1]Fisheries and Oceans Canada, Dartmouth, NS; [2]Temple University,Philadelphia, PA; [3]United States Environmental Protection Agency, Cincinnati, OH

Oil spills in the open sea are dispersed into oil droplets by wave actions under natural and chemically enhanced conditions. Dispersant effectiveness is dependent on environmental factors such as wave conditions. A wave tank has been constructed at BIO to study the effectiveness of chemical dispersant effectiveness as a function of energy dissipation rate. Wave tank experiments have been conducted to investigate wave- and current-driven turbulence effect on chemical dispersion of oil. Total dispersed oil concentration and oil droplet size distribution are strongly dependent on wave types. Interpretation of oil chemistry data is expected to help understanding spatial and temporal trends in toxic response of naturally and chemically dispersed oil under various wave conditions. Dissolved PAH concentrations from the dispersed oil are estimated on the basis of Raoult's law. Aquatic toxicity of the dispersed oil is estimated by toxicity units that are calculated based on dissolved oil and emulsified whole oil PAHs. Research needs in toxicity response of dispersed oil for environmental risk assessments and the selection of on-site remediation activities will be discussed.

Alkyl phenanthrenes, fluorenes, and napthobenzothiophenes are the main components of crude oil that are chronically toxic to fish. P. Hodson[1], C. Khan[1], G. Saravanabhavan[1], L. Clarke[2], S. Brown[1], B. Hollebone[2], Z. Wang[2], J. Short[3], T. King[4] and K. Lee[4] (PL)

[1]Queen's University,Kingston, ON; [2]Environment Canada, Gatineau, QC; [3]National Oceanic and Atmospheric Administration, Alaska; [4]Fisheries and Oceans Canada, Dartmouth, NS

We used "effects-driven" fractionation of Alaska North Slope and Scotian Light crude oil to show that CYP1A induction in juvenile rainbow trout and chronic developmental toxicity to Japanese medaka (blue sac disease or BSD) are associated mainly with 3-ringed alkyl PAH. Of four fractions prepared by low-temperature vacuum distillation (F1-F4), F3 (31% of total carbon in whole oil) contained the highest concentration of PAHs, was the strongest CYP1A inducer, and was chronically toxic, causing the same signs of toxicity as whole oil. Fraction F4 (high molecular weight PAHs) caused moderate CYP1A induction, but was non-toxic. In contrast, F1 and F2, contained mono-aromatics and alkylnapthalenes, did not induce CYP1A in trout, but caused acute narcosis to medaka. These volatile fractions would not persist to cause chronic toxicity. Sub-fractions of F3 were created by cold acetone extraction: F3-1 (extract; rich in PAHs; 35% of total carbon) and F3-2 (wax precipitate; trace amounts of PAHs; 65% of total carbon). The F3-1 extract was a strong CYP1A inducer and highly toxic, while the F3-2 precipitate was not. Normal-phase HPLC separation of F3-1 produced five sub-sub-fractions: F3-1-1 (saturates and monoaromatics), F3-1-2 (alkyl napthalenes and dibenzothiophenes), F3-1-3 (alkyl fluorenes, phenanthrenes, and

napthobenzothiophenes), F3-1-4 (chrysenes, pyrenes), and F3-1-5 (residual material recovered by column backwash). CYP1A induction in trout was caused by F3-1-3 to F3-1-5, but not by F3-1-1 and F3-1-2. The most toxic fraction was F3-1-3, indicating that alkylphenanthrenes, fluorenes, and napthobenzothiophenes, unique to this fraction, were the constituents causing the chronic toxicity of whole oil.

Carbon dynamics, food web structure and reclamation strategies in Athabasca oil sands wetlands (CFRAW). J. J. Ciborowski[1], D. G. Dixon[2], L. Foote[3], K. Liber[4] and J. E. Smits[4] (PL)

[1]University of Windsor, Windsor, ON; [2]University of Waterloo, Waterloo, ON; [3]University of Alberta, Edmonton, AB; [4]University of Saskatchewan, Saskatoon, SK

Wetlands will make up 20-50% of the final reclamation landscape of areas surface mined for oil sands in northeastern Alberta. CFRAW is a collaboration among seven mining partners and five university labs to study effects of mine tailings and process waters on development, health and function of wetland communities expected to form in post-mining landscapes. Our work tests predictions about how quickly wetlands amended with reclamation materials approach the conditions seen in reference wetland systems. Supplementing constructed wetlands with stockpiled peat or topsoil is expected to accelerate succession and community development. The hydrocarbons in tailings (bitumen) and water (naphthenic acids) that occur in wetlands constructed with mine process materials are initially toxic, but may ultimately serve as a surrogate source of carbon once they degrade and/or are metabolized by bacteria. We are assessing the sources, biological uptake, pathways, and movement through the food web of materials used by the biota in constructed wetlands. Studies in progress are evaluating how productivity of new wetlands is maintained. We are monitoring net ecosystem productivity, rates of organic carbon accumulation from microbial, algal, and macrophyte production, and influx of outside materials. We are also comparing rates of leaf litter breakdown and microbial respiration to determine how constituents speed or slow food web processes of young and older wetlands. Stable isotope measurements of carbon and nitrogen in food web compartments indicate which sources are incorporated into the food web as wetlands age; how this influences community development, food web structure and complexity, and the productivity and health of fish, amphibians, and wetland birds. Flux estimates will be combined to determine whether wetlands built with peat amendments can be expected to maintain their productivity and have the potential to ultimately become true peat lands. The research will provide a conceptual model of carbon pathways and budgets to assess how the allocation of carbon among compartments changes as newly formed wetlands mature in the boreal system. Ultimately, we will recommend the materials and strategies most effective and economical in producing a functioning reclamation landscape

Application of PETROTOX in chemical risk assessments of petroleum stream substances. M. Lopez[1], R. McWhinney[1] and T. Stieglitz[1] (PL)

[1]Environment Canada, Edmonton, AB

The PETROTOX spreadsheet model, developed by Conservation of Clean Air and Water in Europe (CONCAWE), is used to estimate the toxicity of complex petroleum mixtures to various aquatic organisms. PETROTOX uses the Target Lipid Model (TLM) and toxic unit theory to provide acute or chronic toxic loadings or dose response data. The PETROTOX model selects the chemical composition and corresponding physical-chemical data from a chemical database included in the model to match an input of the boiling range of each distillation fraction and the hydrocarbon class. A user-defined version (PETROTOX-UDM) requires the physical-chemical properties to be input by the user for each chemical component of the petroleum mixture. The PETROTOX model is easily applied to fuel oils and other heavy petroleum streams. Limitations of the chemical database make the PETROTOX-UDM model better suited for naphthas, gasolines and other light petroleum streams.

Environment Canada is investigating the applicability of the PETROTOX models for chemical risk assessments of petroleum stream substances. Preliminary results indicate that the model is able to reasonably predict toxic loadings of fuels in the order of magnitude range for species of interest. The model may find use during the assessment process as a predictor for petroleum mixtures that lack data on aquatic toxicity if it proves to return reliable results for petroleum streams of known toxicity.

Exploring the biodegradation and toxicity of naphthenic acids present in Athabasca oil sands process-affected waters using simulated wetlands. N. Toor[1], K. Liber[1], M. MacKinnon[2] and P. Fedorak[3] (PL)

[1]University of Saskatchewan, Saskatoon, SK; [2]Syncrude Canada Ltd., Edmonton, AB; [3]University of Alberta,Edmonton, AB

The toxicity of oil sands process-affected water (OSPW) from the Athabasca Oil Sands (AOS) in northern Alberta, Canada, has been shown to be at least partially related to a relatively persistent group of dissolved organic acids known as naphthenic acids (NAs). In this research, we evaluate the potential for the degradation and associated reduction in toxicity of OSPW in wetland environments, a proposed strategy for reclamation at the AOS. Using laboratory microcosms (20.8-L aquaria with a flow-through design) to mimic natural wetlands, we set out to determine if the toxicities of OSPWs generated by Syncrude Canada Ltd. (Syncrude) and Suncor Energy Inc. (Suncor) change as a result of aging and biodegradation over time. Experimental manipulations include two types of OSPW (Syncrude and Suncor), increased nutrient availability (nitrogen and phosphorus enrichment), and short and long hydraulic retention times (40 and 400 days). For the first time, NAs present in OSPW are tracked over the course of one year in order to investigate the relationships between total NA concentrations (quantification using Fourier Transform Infrared Spectroscopy), the degree to which different sub-groups of

NAs are biodegraded (based on 'fingerprinting' using Gas Chromatography Mass Spectrometry), and any potential reduction in OSPW toxicity as measured by the Microtox® bioassay.

Spatial and temporal variability of net ecosystem production in Alberta oil sands wetlands. C. Wytrykush[1] and J. J. Ciborowski[1] (PL)

[1]University of Windsor, Windsor, ON

This research is part of the Carbon Dynamics, Food Web Structure and Reclamation Strategies in Athabasca Oilsands Wetlands (CFRAW) Project. The purpose of my research is to investigate carbon dynamics by measuring net primary production of naturally occurring and constructed wetlands in the oil-sands region of Northeastern Alberta. These wetlands contrast in condition (oil sands process-affected vs. reference), level of sediment organic content (lower vs. higher), and age (younger vs. older). Oil-sands process materials (OSPM) are enriched with several types of compounds including sulphate ions, ammonia, polycyclic aromatic hydrocarbons (PAHs), and naphthenic acids. In high concentrations, these compounds are potentially toxic to aquatic invertebrates. Pilot studies have indicated differences in primary production measured using in situ dissolved oxygen probes. Young OSPM wetlands were generally more productive than reference wetlands of the same age class, but these differences were less distinct in older wetlands. Continuously recording dissolved oxygen probes were deployed in oil sands wetlands throughout the summer of 2006 and 2007. Preliminary analyses demonstrate similar trends. Ongoing analysis will determine seasonal and spatial trends in net ecosystem production in these wetlands and how these are affected by wetland condition, sediment organic content, and age. Results from this study will be linked to estimates of benthic secondary production and invertebrate diversity.

Carbon sources, microbial community production and respiration in constructed oil sands wetlands. C. A. Daly[1] and J. J. Ciborowski[1] (PL)

[1]University of Windsor, Windsor, ON

Carbon sources, microbial community production and respiration were determined in nine northeastern Alberta wetlands subject to oil sands mining. A stable isotope mixing model estimated the proportion of carbon sources (primary production, sediment and petroleum) assimilated by microbes. The proportions of petroleum-derived carbon from oil sands process material (OSPM)-affected wetlands ranged from 62-97%. Bacterioplankton production was quantified by monitoring 3H-leucine incorporation into bacterial proteins. Production and methanogenesis were inhibited by sulphate and/or salinity. Amending wetland sediments with topsoil, a reclamation strategy, did not affect bacterial production or stimulate decomposition. Unvegetated wetland sediments were small net exporters of C and do not appear to be on a trajectory to becoming net sinks in these early stages of development. Overall, microbial functional processes in OSPM-affected wetlands are markedly different from processes in reference-constructed wetlands of equivalent age and do not correspond to those in a mature natural wetland.

The effects of petroleum coke amendments in constructed oil sands wetlands: Trace metal concentrations in pore waters, macrophyte and invertebrate tissues. L. Baker[1], J. J. Ciborowski[1] and M. M. MacKinnon[2] (PL)

[1]University of Windsor, Windsor, ON; [2]Syncrude Canada Ltd., Edmonton, AB

As a by-product of oil sands mining, oil sands operators of Fort McMurray, Alberta, produce over six million tonnes of petroleum coke per year, containing elevated levels of sulphur and trace metals. The use of this waste product to stabilize clay-dominated mine tailings in constructed wetlands is currently being studied as an option for landscape reclamation. Accurate information about the fate of these metals in wetland food web compartments is needed for the sufficient evaluation of site-specific environmental risk assessments. Previous laboratory studies have shown that water leached from this petroleum coke contains potentially concerning levels of V, Ni, Cd, Z, and Mo. Using a field-based microcosm method, we studied the in situ effects of petroleum coke as a sediment amendment in a constructed oil sands wetland. Coke amendments did not significantly increase the concentration of measured trace metals in the sediment pore waters or in associated wetland biota (macrophytic algae *Chara vulgaris*, aquatic snail *Lymnaeidae*, predatory invertebrate Tanytarsus (*Chironomidae*)). These results may reflect the presence of many organic and inorganic constituents found in the wetland sediments and water (peat, naphthenic acids, other dissolved organic carbon species, elevated salinity), which can complex with metals, making them biologically unavailable. Metal concentrations in this constructed wetland were found to be similar to a 30-year-old undisturbed wetland in the area. Trace metal concentrations in the sediment pore waters of this natural wetland were found to be naturally high, exceeding levels dictated by the Canadian Water Quality Guidelines for the Protection of Aquatic Life.

Oil sands as primary succession substrates and the importance of early carbon production on site. J. Hornung[1] and L. Foote[2] (PL)

[1]Petro Canada, Edmonton, AB; [2]University of Alberta, Edmonton, AB

Ecologists rarely have the opportunity to study the growing environment of primary succession substrates. Though examination of primary succession is usually a highly suspect and esoteric undertaking, in the case of Alberta's oil sands, heat-sterilized wetland and aquatic substrate will be placed over hundreds of square kilometres of the boreal landscape as a byproduct of oil extraction processes. We used controlled environments and opportunistic wetland development to examine (a) the survival and growth rates of plants inserted into these new substrates and (b) the volumes of carbon produced in naturally colonized and planted plots on oil sand substrates. We confirmed that although not technically "soil," the produced substrates supported algal and macrophyte production at different rates depending on water quality. Early examinations suggest macrophyte growth and expansion is affected by substrate treatment, and reclamation goals will be achieved earlier where substrate strata are treated with soil amendments.

The growth and photosynthesis of Typha in oil sands process-affected material and water. L. Foote[1] and J. Hornung[2] (PL)

[1]University of Alberta, Edmonton, AB; [2]Petro Canada, Edmonton, AB

Typha (cattail) plants acquire and cycle carbon and nutrients through wetlands. Emergent aquatic plants like cattail contribute substantially to the energy flow in wetlands. The growth and eventual recycling of nutrients captured by aquatic plants like cattail are an important part of natural, healthy wetland ecosystems. Cattail are ubiquitous and satisfy many of the criteria to be used as indicators of wetland integrity. We investigated if cattail growth and carbon accrual were affected by oil sands process materials (OSPM) such as Consolidated Tailings (CT). This research may help reclamation initiatives by determining the effect the constituents of oil sands process material have on aquatic plant growth. This research was conducted at the SUNCOR experimental trenches; six 50 X 10m lined basins, for which we controlled the water inputs. FACTORS: 1) Trench Water: Three filled with natural water (<600 uS) and three filled with oil sands process water (>1200 uS). 2) Soil Type: Cattail planted in different growth medium combinations (into buckets): CT over CT; Soil over Soil; Soil over CT; Soil over Sterilized Sand. MEASUREMENTS: 1) Six rounds of individual leaf length by width of all leaves, 2) Photosynthesis of leaf (Licor 6400 Photosynthesis meter), 3) root and plant biomass at planting and after two years' growth. We observed increased leaf area under oil sands process influence; this could indicate increased carbon accrual above ground. Leaf area data suggest that CT-affected plants are quite productive. The results summarizing the photosynthesis of cattail leaves suggest that oil sands-affected water reduces plant fitness, and therefore could impact overall oil sands reclamation timelines. Conversely, typha grown in soil-capped process-affected material has significantly greater leaf area than those grown in soil-capped sand. This may be explained by elevated ammonia in process-affected material that allows for the greater leaf area, albeit short-term, observed in the above result.

Ecosystem function in oil sands wetlands: rates of detrital decomposition, moss growth, and microbial respiration in oil sands wetlands. C. Wytrykush[1] and J. Hornung[2] (PL)

[1]University of Windsor, Windsor, ON; [2]Petro Canada, Edmonton, AB

Decomposition of dead plant matter is an integral process that partly fuels primary productivity in wetlands. We examined leaf litter breakdown and biomass accrual in 31 reference and oil sands affected (OSPM) wetlands in Northeastern Alberta. The wetlands contrasted in water origin (OSPM vs. reference), sediment origin (OSPM vs. natural), sediment organic content (high vs. low), and age (older vs. younger). We placed mesh bags containing 5 g of dried Typha (cattail) or 20 g of damp moss (=5 g dry mass) in 31 wetlands to monitor the rate at which biomass was lost to decomposition, as measured by changes in dry mass. After one year, moss growth was greatest in younger wetlands with natural sediments. Cattail decomposition was slower in wetlands containing OSPM water than in reference wetlands. Preliminary analysis of respiration rates of flora and fauna

associated with decomposing cattail suggests that the amount of oxygen consumed is unaffected by wetland water source (OSPM vs. reference), sediment source (OSPM vs. reference), level of initial sediment organic content (high/amended vs. low/unamended), or age class (younger vs. older). The data collected from this study will provide information about carbon flow and dynamics in oil sands affected wetlands

The effects of oil sands constituents on the reproductive physiology of fathead minnows (Pimephales promelas). R. Kavanagh[1] (PL)

[1]University of Guelph, Guelph, ON

In the process of extracting bitumen from oil sand, large volumes of fluid tailings are produced. Developers plan to eventually transfer these tailings to mined-out pits and cap them with clean water to create a self-contained aquatic environment. Earlier studies have suggested that oil sand constituents may alter the reproductive physiology of fish. In order to determine if fluid tailings would impair reproduction, 21-day fathead minnow reproduction assays were conducted in northern Alberta. Fathead minnows were held in Gregoire Lake water (reference site) for 14 –21 days to gather baseline data on fecundity, fertility, and hatching success. Fathead minnows were then held in oil sands process-affected waters (OSPW) or Gregoire Lake water (reference) for 21 days. Spawning was reduced in fish that had been held in OSPW and male fish had tubercles that were reduced both in size and number. Follow-up experiments were conducted to identify the oil sands constituents responsible for the reproductive impairment observed in these fish. Collectively, these studies show that oil sands tailings have the potential to affect the reproductive physiology of fish.

Chemically dispersed diesel oil is more toxic to rainbow trout (Oncorhynchus mykiss) than non-dispersed diesel oil. A. Schein[1] and P. Hodson[2] (PO)

[1]University of Prince Edward Island and Canadian Rivers Institute, Charlottetown, PE; [2]Queen's University, Kingston, ON

Diesel oil is a complex mixture that includes polycyclic aromatic hydrocarbons (PAHs). PAHs are toxic, as they are hydrophobic and pass readily from water into fish. Therefore, an oil spill poses a risk to fish if the PAHs enter the water column. Oil normally floats as a slick on top of the water, but if a chemical dispersant is used to clean up the oil spill, compounds from the oil spread throughout the water.

Juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to varying concentrations of water-accommodated fractions (WAF) and chemically enhanced water-accommodated fractions (CEWAF) of diesel oil for 24 hours. CEWAF was made with the dispersant Corexit 9500. The hepatic CYP1A activity of the trout was assessed and found to be consistently high across all CEWAF concentrations, but only the highest WAF concentration produced CYP1A activity greater than water controls.

Larval rainbow trout were chronically exposed to varying concentrations of WAF and CEWAF from egg hatch to swim up. Toxicity was measured by scoring the fish for signs

of blue sac disease (BSD). Mortality was recorded and was less than 20 % in all treatments except for the highest CEWAF concentration, which had 88 % mortality. Sublethal toxicity increased with increasing CEWAF concentration, but only the highest WAF concentration showed elevated signs of BSD. These results indicate that using a chemical dispersant enhances the toxicity of diesel oil to rainbow trout by a factor of 100.

Assessment of metal releases and leachate toxicity of oil sands coke. ***N. Puttaswamy[1] and K. Liber[1] (PO)***

[1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK

Thermal upgrading of oil sands bitumen in Alberta, Canada, produces an enormous volume of coke as a by-product. This large volume of coke is stored on-site and may potentially be integrated into wet landscape reclamation options in the future. However, coke releases trace amounts of metals when it comes in contact with water through leaching processes. Hence the objective of coke leaching was to assess coke leachate water toxicity and perform toxicity identification evaluations (TIEs) on the leachate to determine the cause of toxicity. The leachability of metals from coke was examined over a 45-day period in 2-L glass jars using reconstituted water with a hardness of 300 $mg \cdot L^{-1}$ as CaCO3 at two pH treatments (5.5 and 9.5). Periodically collected samples of overlying water and coke porewater were analyzed for trace metals using ICP-MS. At the end of the 45-day period, toxicity of coke leachate was evaluated using a *Ceriodaphnia dubia* standard three-brood chronic test with survival and reproduction as endpoints. Coke leachate water from both pH treatments had a negative effect on survival and reproduction of *C. dubia*. Metal analysis of leachate water from both pH treatments revealed high concentrations of vanadium in the coke leachate. Coke leachate water will be subjected to several TIE manipulations to identify and hopefully confirm the cause(s) of toxicity.

Distinguishing size from growth rate: Effects of food quality and feeding history on the relative nucleolar diameter of salivary gland polytene chromosomes in <u>Chironomus riparius</u> midge larvae (Diptera: Chironomidae). ***J. P. Martin[1], J. J. Ciborowski[1] and C. Wytrykush[1] (PO)***

[1]University of Windsor, Windsor, ON

Chironomid larvae possess giant chromosomes in their salivary glands. Two laboratory experiments were designed to assess whether or not chromosomal puffing could be used as a reliable indicator of chironomid growth by evaluating the relative nucleolar diameter (RND: ratio of nucleolar diameter/chromosomal width) of puffs in the nucleolar organizer region (NOR). In the first experiment, first instar *C. riparius* larvae were inoculated into treatment jars (n=8) differing in food quality (ground Tetramin™: 0.0, 0.125, 0.25, 0.5, or 1.0 mg/individual/day), while in a second experiment, larvae were

inoculated into one of four treatment jars (n=8) differing in feeding history, following a 2 x 2 reciprocal design. Larvae were fed daily for 14 days, then preserved in chilled Carnoy's solution (3:1 v/v absolute ethanol:glacial acetic acid). Survival was not significantly different between treatments, but food quality and feeding history significantly affected size ($p<0.001$). Salivary glands were excised, squashed, and Giemsa stained. The RND of each larva was determined using image analysis of video-projected slide mounts viewed under a compound microscope (analysis in progress). If RND reflects an individual's most recent feeding history, instantaneous growth could potentially be determined from a single field-collected sample, facilitating the estimation of production and the assessment of aquatic ecosystem condition. Field samples are currently being collected in oil sands-affected wetlands.

PETROTOX talks toxicity. ***M. Lopez[1], R. McWhinney[1] and T. Stieglitz[1] (PO)***
[1]Environment Canada, Edmonton, AB

The PETROTOX spreadsheet model, developed by Conservation of Clean Air and Water in Europe (CONCAWE), is used to estimate the toxicity of complex petroleum mixtures to various aquatic organisms. The PETROTOX model is easily applied to fuel oils and heavy petroleum streams. A user-defined version is also available (PETROTOX-UDM). This model requires the physical-chemical properties needed for the determination of equilibrium concentrations and lipid concentrations (i.e., Henry's law constant, octanol-water partition coefficient, aqueous solubility and molecular weight) to be defined by the user rather than obtained from the chemical database. Limitations of the database make the PETROTOX-UDM model better suited for naphthas, gasolines and light petroleum streams. The models have the potential to assist Environment Canada in the chemical risk assessment of petroleum stream substances by providing toxicity estimates for streams with few published aquatic toxicity data.

Toxicity of dispersed MESA crude oil to early life stages of herring (Clupea harengus).
S. McIntosh[1], K. McAlindon[1], S. Courtenay[2] and P. Hodson[1] (PO)
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Crude oil is transported across several herring fishing zones along the Atlantic coastlines of Canada and the United States. In the event of a spill, efforts to minimize ecological damage may include chemical dispersion of oil into the underlying water to protect shorelines and wildlife from floating oil. While dispersion may be appropriate in some instances, its potential risks and benefits depend on the species living in the contaminated area. For larval herring, the toxicity of dispersed crude oil is about 100 times greater than that of water-accommodated (mechanically-dispersed) crude oil fractions. Furthermore, dispersed oil may be differentially toxic during certain stages of embryonic herring development. It is important to improve our understanding of dispersed oil's toxicity to herring to ensure that the current guidelines for dispersant use are applicable to spills in herring habitats. By identifying the stages of herring development that are most sensitive

to dispersed oil, we aim to determine the times of year when exposure to dispersed crude oil would be most detrimental to herring recruitment. The lowest dispersed hydrocarbon concentration that elicits a negative response during herring development will then be compared to time- and concentration-dependent models of oil dispersion. This comparison will help to characterize the spatial conditions in which developing herring would likely encounter harmful concentrations of hydrocarbons after a spill is dispersed, thereby summing the temporal and spatial information that is necessary to make sound decisions on dispersant use in herring habitats and fishing zones.

Effect of produced water on feeding and metabolism of Atlantic cod (Gadus morhua).
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Co-existence of the oil industry activities with fish, fish habitat, and commercial fishing leads to concerns regarding potentially harmful cumulative effects of waste products on marine organisms around production sites. Environmental parameters have been shown to induce changes in metabolic capacities, feeding, and digestive physiology of fish, which in turn could have drastic consequences on growth or health status of fish populations. We have investigated the effects of produced water (PW) on feeding and metabolism of Atlantic cod by exposing fish to 0,100 $mg \cdot L^{-1}$ (x10,000 PW dilution) or 200 $mg \cdot L^{-1}$ (x500 dilution) of PW for 76 days. Food intake and mean weight were monitored throughout the experiment and serum lipids (cholesterol, triglycerides) and metabolites (lactic acid, glucose and proteins), as well as gene expression of a brain appetite-regulating factor (neuropeptide Y, NPY) were measured at the end of the experiment. No significant differences were observed in weight gain or food intake between the three groups of fish. Moreover, serum metabolites and NPY expression appeared to remain unchanged between groups. Comparative measurements of whole blood fatty acid profiles in plasma are being completed. Our preliminary results suggest that PW does not significantly affect feeding and metabolism in cod.

The use of stable isotopes to examine oil sands-derived polycyclic aromatic compounds and utilization by primary consumers.
A. Farwell[1], D. Vukosavljevic[1], B. Butler and D.G. Dixon[1] (PO)
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Polycyclic aromatic compounds (PACs) are a group of naturally occurring oil sands constituents present in the process water and concentrated in the mature fine tailings (MFTs) that are generated during the extraction of bitumen from oil sands in Alberta, Canada. When MFTs are used in subsequent on-site reclamation, benthic invertebrates are exposed to elevated levels of PACs. To better understand the importance of PAC

derived carbon as an energy source in oil sands aquatic reclamation, stable isotope analyses were used as a tool to trace oil sand C and N sources in microbes and primary consumers in the laboratory. In this study, blackworms *(Lumbriculus variegates)* were reared for six weeks on either whole sediment from different oil sands reclamation wetlands containing elevated levels of PACs, or on microbes cultured on oil sands-derived PAC extracts. The nitrogen isotope signatures of *L. variegates* were similar to that of the sediment within four weeks of exposure, whereas carbon isotope signatures of *L. variegates* were ~2 ‰ 13C enriched relative to the sediment. The rapid turnover rate for nitrogen suggests that tracing stable nitrogen isotopes may be a useful tool for determining exposure of aquatic organisms to oil-sands-processed material via routes such as groundwater contamination.

The effect of modifying factors on the toxicity of oil sands reclamation sediments to medaka embryo development. A. Farwell[1], A. Lau[1] and D.G. Dixon[1] (PO)

[1]University of Waterloo, Waterloo, ON

Alkylated polycyclic aromatic compounds (PACs) and naphthenic acids (NAs) are naturally occurring oil sands constituents. These compounds are found at elevated levels in aquatic oil sands reclamation in northern Alberta, Canada. Oil sands-derived alkylated PACs and natural oil sands sediments have been shown to induce signs of blue sac disease (BSD) in various fish species. Fish embryos exposed to an oil sands-derived PAC mixture and ultraviolet (UV) irradiation caused slight phototoxicity. For fish embryo development endpoints, NAs are less toxic than PACs. However, it is unclear if low levels of NAs, a natural surfactant, influence the toxicity of PACs. The objective of this study is to assess the effects of oil sands aquatic reclamation sediments and modifying factors (exposure to NA and UV) on the development of Japanese medaka embryos. Medaka embryos were exposed to sediments from a variety of oil sands aquatic reclamation strategies as well as mature fine tailings. Oil sands reclamation sediments were mixed with water containing different levels of NAs (at or below the lowest-observed-effect concentration) prior to exposure to medaka embryos. For each sediment treatment, replicates (3) were exposed to simulated solar radiation with and without UV. At hatch, embryos were assessed for deformities and hatch length. Consideration of these modifying factors is important in terms of reclamation options that may reduce potential enhanced toxicity.

The use of nutrient addition as a wetland reclamation strategy in the oil sands region of Alberta, Canada. M. Boutsivongsakd[1], A. Farwell[1], H. Chen[1], N. Heerkens[1], B. Warner[1] and D.G. Dixon[1] (PO)

[1]University of Waterloo, Waterloo, ON

The mining and extraction of bitumen from the oil sands region in Alberta, Canada, generates a large volume of process-material that must be reclaimed. The use of oil sands process-material (OSPM) to create wetlands and shallow lakes is a challenge, as this material may have a strong impact on the rate and type of aquatic faunal and floral colonization. In this case, the quality of the substrate may be poor due to physical (particle size, organic content) and/or chemical (elevated levels of polycyclic aromatic compounds, PACs) characteristics. Due to the potential chronic effects of PACs, it is

advantageous to construct wetlands that promote optimal sedimentation to rapidly confine potentially toxic OSPM used as an initial substrate. One approach involves nutrient addition. The objectives of this study are to: 1) examine the influence of nutrient addition on plankton and periphyton production; 2) examine the impact of maximum sedimentation on invertebrate and macrophyte colonization. To examine the effects of nutrient addition, mesocosms were set up at three different reclamation sites along a gradient of naphthenic acid concentration in the water. Naphthenic acids are a complex mixture of naturally occurring oil sands constituents that are toxic to aquatic organisms. For each site, mesocosms were lined with three different substrates (mature fine tailings/sand, reclamation soil, sand). Nutrients were added to the mesocosms to create oligotrophic (no addition), mesotrophic (low level addition), and eutrophic (high level addition) conditions for each water treatment. Preliminary results will examine planktonic and periphytic production/sedimentation.

Reproductive performance and neonatal development of tree swallows (Tachycineta bicolor) on reclaimed mine sites on the Athabasca oil sands. H. Keshwani[1], B. Hersikorn[1] and J. E. Smits[1] (PO)

[1]University of Saskatchewan, Saskatoon, SK

Reclaimed wetlands created from mine tailings on the Athabasca Oil Sands were compared for their ability to support populations of tree swallows, sentinels representing upper trophic level feeders. Swallows breed in nest boxes provided on the sites of interest, and raise their young while feeding on flying insects emerging from local wetlands. Stressors, including contaminants accumulated through the diet, may affect a bird's health. We examined two experimental wetlands differing in age: 15 years old and three years old.

Health of the birds was evaluated through reproductive performance and neonatal development. Reproduction was measured by clutch size, egg mass, hatchability, and offspring survival. Neonatal development was evaluated through body measurements taken on 12-day-old nestlings.

Reproductive success of the adults, and clutch size, did not differ between the sites although initiation of breeding was earlier on the young site. Neonatal development was different between sites although body mass was not. Accelerated tail- and wing-feather maturation was observed on birds from the younger reclamation site, while the skeletal size was greater on the mature site. These findings may be due to different dietary, nutritional, and/or endocrine modulating substances moving through the food chain. Altitude and vegetation cover was different between the sites, which could influence the insect density and diversity as well.

Spatial variation in microbial respiration in oil sands-affected and reference wetlands measured using gas elution from sediments. J. Gardner Costa[1] (PO)

[1]*University of Windsor, Windsor, ON*

Wetlands have been seen as potential carbon sinks to mitigate increasing atmospheric, carbonaceous, greenhouse gases. Recent oil sands mining and extraction in northeastern Alberta have displaced many wetlands from the area. As part of its reclamation strategy, the oil sands industry has begun to reconstruct these wetlands using oil sands process material (OSPM), with the intention of restoring pre-mining wetland function. However, oxidation of detritus and other organic matter by the wetland microbial community may slow carbon accumulation, and thus contribute to greenhouse gas emissions instead of reducing them. Microbial production of carbon dioxide and methane resulting from respiration eluting from sediments was measured in submergent vegetation and open water areas of 10 wetlands for 0, 24 and 72 hrs. 500-mL polyvinyl-chloride microcosms were used to trap and determine the concentration and volume of these gases rising from the sediments. Gas chromatography will be used to identify and quantify the gas collected. I expect that younger wetlands will elute more gases than older wetlands as microbial species succession will bring additional organisms so that chemoheterotrophs are not as dominant. As well, OSPM-affected wetlands should elute more gases than reference wetlands as succession may cycle longer in the OSPM wetlands. Measuring gaseous carbon fluxes in oil sands wetlands will establish whether young wetlands are suitable carbon sinks or on trajectory to becoming them.

Municipal and Industrial Effluents

Session Co-chairs/Présidents: Wendy Krkosek and Joanne Parrott

Stereospecific analysis of wastewater-derived beta-blockers, selective serotonin re-uptake inhibitors, and salbutamol by chiral high-performance liquid chromatography-tandem mass spectrometry. E. McClure[1], C. Wong[1] and S. MacLeod[1] (PL)

[1]*University of Alberta, Edmonton, AB*

A reversed-phase chiral liquid chromatography-tandem mass spectrometry (HPLC-MS-MS) method was developed to measure enantiomer fractions (EF) and concentrations of pharmaceuticals in wastewater. Enantiomer resolution of six β-blockers (atenolol, metoprolol, nadolol, pindolol, propranolol, and sotalol) along with two selective serotonin re-uptake inhibitors (citalopram, fluoxetine) and one β2-agonist (salbutamol) was achieved with the Chirobiotic V stationary phase. Analyte recovery averaged 86% in influent and 78% in effluent with limits of detection ranging from 0.2 to 7.5 $ng \cdot L^{-1}$. These results represent an improvement in wastewater EF measurement for atenolol, metoprolol and propranolol as well as the first EF measurements of citalopram, fluoxetine, nadolol, pindolol, salbutamol and sotalol in wastewaters. Changes in EF through treatment indicate biologically-mediated stereoselective processes were likely occurring during

wastewater treatment. Stereospecific analysis can indicate these changes and add specificity to risk assessment, especially where differential toxicity exists between enantiomers.

Membrane filtration of organic micropollutants from natural waters. A. Comerton[1], R. Andrews[1] and D. Bagley[2] (PL)

[1]University of Toronto, Toronto, ON; [2]University of Wyoming, Laramie, WY

The detection of trace levels ($ng \cdot L^{-1}$) of endocrine-disrupting compounds (EDCs) and pharmaceutically active compounds (PhACs) in the environment has brought increasing concern over their potentially adverse ecological and human health impacts.

Conventional water treatment processes do not generally provide effective removal of EDCs and PhACs. As such, there is a need to investigate the ability of advanced treatment processes, such as membrane filtration, to remove these emerging contaminants. This study examined the ability of high-pressure membranes to remove twenty-two EDCs and PhACs from lake water, membrane bioreactor effluent and laboratory-grade water (Milli-Q®). The influence of water matrix and compound properties on removal was also studied.

A bench-scale membrane filtration system incorporating three stainless steel crossflow cells (Sepa CF II) was used to investigate removal by nanofiltration (NF) and reverse osmosis (RO) membranes. Removal efficiencies were found to be a function of the compound, membrane, and matrix properties. Rejection from lake water and Milli-Q® water was observed to be strongly correlated with compound log Kow. Therefore, the more hydrophobic the compound, the higher the rejection. Similar correlations were seen in a previous adsorption bottle test study by the authors. As such, it is hypothesized that adsorption mechanisms are contributing, at least in part, to compound retention by the membrane. In addition, better compound rejection was observed with natural waters than with Milli-Q® water. It is hypothesized that foulants present in the natural waters modify the membrane surface characteristics and membrane structure leading to improved rejection. Additional research is required to further identify the underlying mechanisms responsible for removal.

Laboratory calibration and field deployment of a passive sampling device for time-weighted average concentrations of pharmaceuticals and personal care products in wastewater and surface water. P. Sudhir[1], C. Wong[1] and S. MacLeod[1] (PL)

[1]University of Alberta, Edmonton, AB

Aqueous sampling rates for 25 common pharmaceuticals and personal care products (PPCPs) were determined on the commercially available passive Polar Organic Chemical Integrative Sampler (POCIS). To date, POCIS calibration has only been carried out for a few other compounds. Uptake rates were 0.040 to 2.462 L/d under flowing water conditions, while quiescent conditions resulted in uptake rates between 0.016 and 0.223 L/d. The uptake of many PPCPs appears to be boundary-layer controlled, as indicated by higher sampling rates under flowing conditions. Sampling rates were corrected for

analyte dissipation rates, and some correlations with octanol-water partition coefficients were noted. Field deployments of POCIS in wastewater effluent and surface waters provided time-weighted average concentrations, derived from laboratory calibrations, which were consistent with regular grab samples. This work indicates that POCIS can be used as a quantitative tool for measuring time-weighted average concentrations of PPCPs in the aquatic environment.

Fate and effects of pharmaceutically active compounds in a model biofilm system. W. Krkosek[1], M. Van Dyke[2], P. Huck[2] and G. Gagnon[1] (PL)

[1]Dalhousie University, Halifax, NS; [2]University of Waterloo, Waterloo, ON

Pharmaceutically active compounds (PhACs) are designed to resist biodegradation and to elicit a biological response. There is a potential risk that PhACs could cause toxic and multi-generation effects in non-target organisms due to continuous low-level wastewater discharge. Little work has been completed to date on the impacts of PhAC exposure on naturally occurring bacteria and biofilms, and the additive or synergistic effects due to exposures to mixtures of PhACs. This research presents the development of a model batch reactor system used for the assessment of biofilm and suspended bacterial toxicity due to PhAC exposure, and the assessment of PhAC fate within the model system. A model riverine biofilm system was developed to assess both the short-term physiological impacts and the long-term shifts of bacterial communities following PhAC exposure. The microcosm set-up was based upon an Environment Canada biological sediment toxicity test method. Within this model system, both mixtures and individual PhACs were investigated from three different pharmaceutical classes. The effects of PhACs on bacterial populations were evaluated by monitoring live/dead cell counts, ATP, and selective media plate counts. The fate of PhACs within the model system was evaluated by monitoring biodegradation, photolysis and adsorption.

Changes in metallothionein levels in freshwater mussels exposed to urban wastewaters: Effects from exposure to heavy metals? F. Gagné[1], C. Gagnon[1], P. Turcotte[1] and C. Blaise[1] (PL)

[1]Environment Canada, Montreal, QC

Municipal effluents are complex mixtures of compounds such as heavy metals, aromatic and aliphatic hydrocarbons, and micro-organisms, and they are released in aquatic ecosystems. The purpose of this study was to verify whether changes in metallothioneins (MT) were associated with the accumulation of labile metals in tissue of freshwater mussels exposed to the dispersion plume of a major municipal effluent. Mussels were placed in experimental cages deployed at sites 1.5 km upstream, 8 km downstream and 12 km downstream of the outfall of a major, primary-treated municipal effluent in the St. Lawrence River (Québec, Canada). Mussels were analyzed for MT and labile zinc levels in their gonads, gills and digestive glands. Lipogenic enzyme (isocitrate and glucose-6-

phosphate dehydrogenase) and arachidonic acid cyclooxygenase (COX) activities were also measured in gonad and gill tissues. Although MT was induced in all the tissues examined, the results showed that labile zinc levels were significantly reduced in gill and gonad tissues, with an increase observed only at the 12 km downstream site in the digestive gland. COX activity was readily induced in gills and gonads. Glucose-6-phosphate dehydrogenase activity was reduced at both downstream sites, but isocitrate dehydrogenase activity was significantly induced at the farthest (12 km) site. Analysis of covariance revealed that MT levels in gills were more influenced by COX activity than by distance in the dispersion plume and were negatively correlated with labile zinc levels. In conclusion, MT induction was inversely related to the levels of labile zinc but positively so with the inflammation biomarker COX. Hence, the induction of MT in mussels exposed to the municipal effluent of a large city appears to be associated with either inflammatory processes or as compensation for the loss of labile essential metals. We propose that the simple and complementary parameters of labile zinc and COX evaluations be used to link MT induction with divalent heavy metal exposure or inflammation/oxidative stress in environmental studies dealing with various types of contaminants in such complex contaminant mixture effluents.

Impacts of Halifax Harbour marine pollution on immunomodulation in blue mussels (Mytilus edulis* and *Mytilus trossulus*), endocrine disruption of key predator populations (*Nucella lapillus*), and resultant effect on intertidal community structure.

S. Bard*[1] *and C. Coray*[1] *(PL)

[1]*Dalhousie University, Halifax, NS*

The impact of municipal marine effluent on ecosystem health was assessed along a pollution gradient in Halifax Harbour, NS, Canada, by examining biomarker immune function in mussels (*Mytilus edulis* and *Mytilus trossulus*), endocrine disruption in dog whelks *(Nucella lapillus)* and periwinkles *(Littorina littorea)*, and resultant effects on intertidal community structure. Intertidal diversity studies revealed decreased abundance of community-structuring macrophytes and increased abundance of filter-feeding mussels and barnacles, leading to an overall reduction in species richness at polluted sites. Mussels sampled at sites with lowest intertidal biodiversity displayed impaired immune function in the form of reduced phagocytic activity and reduced production of hydrogen peroxide by cells of the immune system, when compared to mussels from a control site. Halifax Harbour sites displayed high levels of tributyltin (TBT)-mediated endocrine disruption in dog whelks (imposex) and periwinkles (intersex). Periwinkles can tolerate higher TBT exposure and can be found where key predator dog whelks have been extirpated because of TBT contamination. Periwinkle intersex indices corresponded with dog whelk imposex indices where dog whelks were found, and were high where dog whelks were absent. Dog whelks were absent at sites with the lowest intertidal species richness. Municipal marine pollution is a complex mixture of contaminants, making it

difficult to choose endpoints with which to assess effects of exposure on the ecological health of the receiving environment. We believe the solution is to monitor health at the level of the ecosystem, population, and organism down to the cellular and molecular realms in order to obtain a full understanding of effects.

Fathead minnow life-cycle exposure to the β-blocker propranolol. J. Parrott[1] and V. Balakrishnan[1] (PL)

[1]Environment Canada, Burlington, ON

Propranolol is a human pharmaceutical B-blocker used to control heart rate and blood pressure. It has been detected in municipal wastewater effluents in North America at concentrations of low $ng \cdot L^{-1}$ to low $\mu g \cdot L^{-1}$. To assess the potential of this compound to affect fish, fathead minnows were exposed for a lifecycle in a flow-through system to propranolol concentrations of 0, 30, 100, 300, and 1,000 $ng \cdot L^{-1}$ (nominal). Females exposed to 30, 100, 300 $ng \cdot L^{-1}$ were shorter in length and weighed 20 % less than control females. The trend was similar for female fish exposed to 1,000 $ng \cdot L^{-1}$ propranolol, but the changes were marginally significant. No significant changes in weights or lengths were seen in male fathead minnows. There were no significant changes (from control fish) in condition factor, gonadosomatic index, liver-somatic index or sex characteristics in propranolol-exposed male or female fish. Despite their small size, female fathead minnows from propranolol exposures produced as many eggs or more eggs than control fish. Fish exposed to 100 $ng \cdot L^{-1}$ propranolol produced fewer eggs than control fish. Egg production was variable and showed no trends or dose-response with propranolol concentration. Currently we are repeating the lifecycle with a wider range of concentrations (1 to 10,000 $ng \cdot L^{-1}$) to validate the fish exposure concentrations, to assess whether the growth reductions are repeatable, and to determine the LOEC and NOEC. The study is important as it assesses the potential for effects in fish exposed to environmentally-relevant concentrations of propranolol during all critical windows of exposure in their lifecycle.

Integrating environmental effects monitoring with P2 plans: the City of Ottawa's municipal wastewater facility as a case study. B. Kilgour[1], B. Dowsley[1], W. Jackson[2], C. Portt[3] and D. Robertson[2] (PL)

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The City of Ottawa recently adopted environmental effects monitoring as a component of its Pollution Prevention Plan for its municipal wastewater facility. Fish communities downstream of the final discharge are diverse and include sturgeon, walleye, channel catfish, and a variety of other species. The benthic invertebrate community lacked sphaeriid clams during the first year of study (2005), but numbers of clams increased in the near-field of the effluent plume one year later. Short-term toxicity tests with raw final effluent have been non-toxic to sphaeriid clams. The transient nature of the benthic community effect demonstrates the need to conduct follow-up studies to confirm the degree and nature of effects. These various studies have benefited the P2 Plan in that

they have demonstrated no negative biological effects on the Ottawa River attributable to total residual chlorine in the effluent, while the specific effluent-related causes of the observed modest impairments require more study.

Toxicity regulation of RLW effluent from Ontario CANDU stations – Past, present and future. D. Rodgers[1] (PL)

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The radioactive liquid waste (RLW) systems in Ontario's CANDU nuclear generating reactors collect, segregate, monitor, process (as necessary) and discharge the water from numerous drains within the radioactive zones of the station. Prior to 1989, RLW effluent was analyzed for radioactivity prior to pump-outs, but was not regulated or monitored for toxicity or conventional water quality parameters, except for pH. However, toxicity testing was among the parameters specified when RLW was classified as a "Process Effluent" under the Ontario's Municipal and Industrial Strategy for Abatement (MISA) regulations. In acute toxicity tests, RLW effluent from all stations was intermittently toxic to rainbow trout and *Daphnia magna*. Despite extensive parallel chemical analysis, no single or combination of inorganic or organic compounds was consistently related with toxicity. Our studies soon shifted to individual stations, as substantial differences were apparent in the toxicity of RLW effluents among stations. There were also differences in the toxicity of effluent from different sources within stations, with metals, hydrazine and laundry waste among the principal toxic components. Although specific treatment systems were designed for each station, RLW effluent remained sporadically toxic and a rapid toxicity test (Daphnia IQ) was used to screen each effluent tank before release. In the last few years, the frequency of screening failures decreased to less than 5%, and all stations now use chemical analysis rather than rapid toxicity testing to screen RLW effluent before discharge. However, MISA regulations still require periodic toxicity testing (monthly or quarterly) of all "Process Effluent." With new requirements for nuclear power generation on the horizon, the lessons learned from this testing will be reviewed to provide a basis for minimizing both treatment costs and environmental effects of RLW effluent in new stations.

What kind of fish health-effects might be associated with landfill leachate: A preliminary study on the Robin Hood Bay landfill in Newfoundland. A. Mathieu[1], J. Hanlon[1], B. French[1], L. Fancey[2], C. Andrews[2], A. Perry[2] and J.F. Payne[2] (PO)

[1]Oceans Limited, St. John's, NL; [2]Fisheries and Oceans Canada, St. John's, NL

The main source of drainage from the large Robin Hood Bay landfill is a small brook, Skerries Brook, which flows through the landfill and empties into the Atlantic Ocean. Preliminary acute and sub-acute toxicity studies were carried out on selected fish and invertebrates exposed to water from Skerries Brook, collected near its point of entry into the ocean. Water for the acute toxicity studies was collected during summer, spring and autumn. Exposure of marine species was carried out with water adjusted for salinity. The

undiluted water was highly toxic, with trout, salmon and cunner dying within a few hours of exposure and mussels and sea urchins within four days or less. Regarding sub-lethal effects on trout, the diluted water was shown to have potential for disturbance to neural/neuromuscular systems (acetylcholinesterase), physiological and cellular functions (mixed-function oxygenase), and endocrine function (vitellogenin). It was also observed in parallel studies with trout and codfish larvae that leachate from garbage bags is a source of MFO enzyme-inducing chemicals. Since garbage bags are a major source of plastic in landfills, the chronic toxicity potential to fish of garbage-bag leachate, as well as other plastic leachate, should be investigated. (Supported by Environment Canada's NAACAP program and DFO).

Wild fish exposed to municipal wastewater effluents in a small receiving environment.
G. Tetreault[1], M. McMaster[1], J. Bennett[1] and M. Servos[2] (PO)
[1]Environment Canada, Burlington, ON; [2]University of Waterloo, Waterloo, ON
An emerging issue in Canada involves the effects of municipal wastewater effluents (MWWE) in the aquatic receiving environments. MWWE, or sewage, is a mixture of household waste, ammonia, inorganic chloramines, textile mill effluents (TMEs), nonylphenol and its ethoxylates, and pharmaceuticals and personal care products (PPCPs), all of which have been detected in environmental samples. Previous studies by Environment Canada on Wascana Creek in Regina, Saskatchewan, Canada, have determined that during the winter low flow periods, the creek is 100% treated sewage. This creek does not receive any other major discharges, making it a simpler model system to understand. With these studies, we are interested in determining whether MWWE affects normal reproductive development in fish in Canada, and if so, does impaired reproductive health influence the survival of fish populations and alter the fish community? In 2005 and 2006, field studies assessed sentinel fish responses in terms of growth (condition factor), reproduction (in vitro sex steroid biosynthetic capacity, gonadosomatic indices, histology) and survival. Comparisons were made in fish collected upstream and downstream of the City of Regina's Wastewater Treatment Plant. An abundant species in this system is the fathead minnow *(Pimephales promelas)*. Male fathead minnow collected downstream of the sewage discharge were smaller, had reduced condition and larger liver somatic index when compared to male fish collected upstream of the City of Regina. These males also had significantly lower scores of secondary sexual characteristics (fewer nuptial tubercles, little or no development of the dorsal pad, and the lack of presence of a dorsal fin dot). Female fish had significantly higher gonadosomatic and liver somatic indices relative to the reference fish. This species is commonly cultured in laboratories for bioassay exposures to evaluate the reproductive effects of industrial effluents and endocrine active compounds. Comparisons will be made between the responses of wild fish from this study to those of fish exposed to MWWE or EDCs in controlled laboratory experiments.

Total fluorine analysis of fish samples using a novel Combustion Ion Chromatography approach. J. Khim[1], J. Giesy[1], P. Jones[1] and J. Naile[1] (PO)

[1]University of Saskatchewan, Saskatoon, SK

Perfluorinated compounds (PFCs) are persistent and widely distributed in the environment. Accurate and precise measurement is essential for effective decisions regarding the production and use of PFCs. The methods for identifying and quantifying PFCs are relatively new and changing rapidly. Because of a lack of robust standards and analytical methods, a large portion of organic fluorine in a sample may be unidentified. Here, for the first time, we report a mass balance approach for total fluoride analysis of fish samples. A Combustion Ion Chromatography method was developed to allow for accurate determination of total fluorine. Samples were fractionated into organic and inorganic factions by use of solid phase extraction. These fractions were analyzed for total fluorine, organically bound fluorine, and inorganic fluorine (fluoride). Using a liquid chromatography tandem mass spectrometer, we were able to determine how much known PFCs such as perfluorooctanesulfonate and perfluorooctanoic acid contribute to the amount of total fluorine found in a sample. A large portion of the organic fluorine found in fish tissue still remains unknown, suggesting the presence of other, yet-to-be-discovered fluorinated acids.

Environmental Effects Monitoring

Session Co-chairs/Présidents: Simon Courtenay and Les Rutherford

Regional reference variability in EEM fish effect endpoints in white and longnose suckers in Lake Superior. B. Fraser[1], D. Farara[1] and N. Ali[2] (PL)

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As part of EEM, "effects" on fish are determined through the comparison of various biometric endpoints in a control-impact paradigm. It is unclear, however, whether EEM studies completed to date have adequately defined natural reference variability in these endpoints. Anecdotal evidence from a number of surveys suggests that this may not be the case. The question arises therefore: Is it possible to assess effects within an assessment framework based on the "different from reference" paradigm when "reference" has not been adequately defined?

Although the multiple or regional reference-area approach has been embraced for the EEM benthic survey, it has to date not been similarly embraced for the fish survey. Yet, collection of fish from multiple areas may be the most effective way to assess potential mill-related effects, as it offers the greatest statistical power to detect a meaningful difference between a reference area and an exposure area. Moreover, with multiple reference areas, if significant differences between exposure and reference fish from multiple areas are found, it is likely a relatively strong indication that the exposure fish do differ in a meaningful way from reference.

In conjunction with the execution of fourth-cycle EEM fish survey programs at mills on Lake Superior, and with assistance from Environment Canada (Ontario Region) to collect data from additional reference sites, white sucker and longnose sucker were collected from several (5) reference areas with the expressed purpose of assessing regional

reference variability in key EEM fish survey effect endpoints. In this presentation, the results of this regional reference fish study are provided and the implications for the interpretation of fish survey data, and the assessment of mill-related effects, are discussed.

Examining the response of white suckers (Catostomus commersoni) after pulp mill shutdown and restart. ***L. Bowron[1], K. Munkittrick[1], M. McMaster[2], G. Tetreault[2] and M. Hewitt[2] (PL)***

[1]University of New Brunswick Saint John, Saint John, NB; [2]Environment Canada, Burlington, ON

The impacts of pulp mills effluents on fish and fish habitat have been studied at Jackfish Bay, Lake Superior, Ontario, Canada since the late 1980's. Jackfish Bay has received the effluent from a large bleached kraft pulp mill (BKM) in Terrace Bay, ON, since 1948. The site is isolated from other sources of contamination, industrial or municipal effluents. Ongoing studies conducted since 1988 showed that white suckers *(Catostomus commersoni)* had a number of reproductive alterations, including delayed maturity, decreased gonadal size, decreased expression of secondary sexual characteristics, and depressed levels of circulating steroids. Reductions in gonad size and steroid levels persisted until the pulp mill closed its hardwood line in the late fall of 2005 and ceased all effluent discharge from its softwood line from February 2006 to September 2006. Fish were collected in the spring and fall of 2006 during shutdown, and then again eight months after the mill reopened. Relative to their performance as measured with the previous 17 years of data, males recovered quickly after mill closure, as did steroid hormone levels in females. Persistent whole-organism responses in females may be related to a time lag associated with recovery of their egg production. Continued studies will evaluate these questions.

Interpreting effluent effects on fish when the magnitude of effect changes with size or age of fish. ***R. Lowell[1] and B. W. Kilgour[2] (PL)***

[1]Environment Canada, Saskatoon, SK; [2]Stantec Consulting Ltd., Ottawa, ON

The National Environmental Effects Monitoring (EEM) Program evaluates the effects of metal mining and pulp and paper mill effluents using field surveys of receiving-water fish and benthic invertebrates. Four of the five core fish survey endpoints are analyzed via Analysis of Covariance (ANCOVA). This method of analysis is used to factor out the influence of size (weight or length) or age on the response variable being measured. For EEM data collected over the last decade, effluent effects are most often fairly constant within a population for fish of different sizes or ages (this usually occurs for well over half of the comparisons being made). Put another way, within a given adult population, big fish and small fish most often respond similarly. In statistical terms, the exposure and reference area slopes in the ANCOVA can be treated as being parallel for these comparisons. For a sizeable proportion of the data, however, the exposure and reference

area slopes are significantly different (termed a significant interaction). That is, the magnitude of effect changes with increasing size or age of the fish. This can be a quite important effect of effluent exposure, but interpretation can be difficult, particularly when making decisions on whether the effect is great enough to require further study or corrective action. We present a new methodology (currently under investigation) for analyzing and interpreting such effects, based on maximum observed deviations in regression lines.

Metabolites of chlorinated syringealdehydes and vanillins in fish bile as biomarkers of exposure to bleached hardwood and softwood pulp effluents. ***J. Roberts[1], S. Goudey[2], T. Tarpey[3] and D. Birkholz[1] (PL)***

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Chlorophenolic compounds are produced during the bleaching process in pulp mills by the chlorine degradation of lignin. Chlorinated syringaldehydes and vanillins are found in bleached kraft mill effluent. Chlorinated syringaldehydes are associated with hardwoods, and chlorinated vanillins are associated with both hardwoods and softwoods. The concentration of conjugated phenolic compounds in fish bile can be very much greater than their concentration in the surrounding water, suggesting that the quantification of metabolites in the bile can provide a marker of exposure to very low, and even fluctuating, levels of aquatic pollutants. Modern-day bleaching mills use chlorine dioxide bleaching, and as a consequence, most chlorinated phenolic compounds present in effluent are at low concentrations or not detected. The exception is chlorinated syringealdehydes and vanillins which are found in the 100's of $ng \cdot L^{-1}$ range. We intraperitoneal-injected large rainbow trout with solutions of the following chemicals in corn oil: 5, 6-dichlorovanillin, 6-chlorovanillin, 2-chlorosyringaldehyde, and 2, 6-dichlorosyringaldehyde. Following depuration for four days, fish were euthanized and the bile sampled. Bile samples were analyzed and the following metabolites identified: 6-chloro-4-hydroxy-3-methoxy benzyl alcohol (6-chlorovanillin); 5,6-dichloro-4-hydroxy-3-methoxy benzyl alcohol (5,6-dichlorovanillin); 2-chloro-3,5-dimethoxy-4-hydroxy-benzylalcohol (2-chlorosyringealdehyde) and 2,6-dichloro-3,5-dimethoxy-4-hydroxy-benzylalcohol (2,6-dichlorosyringealdehyde). In order to assess whether metabolites of chlorinated syringealdehydes and vanillins would be useful in assessing BKME obtained from hardwood and softwood operations, we exposed large fish to effluent for a period of five days and analyzed the bile for these metabolites. We report on our findings.

Can energy intake modify fish reproductive impacts of pulp and paper effluent exposure? ***M. van den Heuvel[1], M. Landman[2], M. Finley[3] and D. West[4] (PL)***
[1]University of Prince Edward Island, Charlottetown, PE; [2]Scion, Rotorura, NZ; [3]Canadian Rivers Institute, Charlottetown, PE; [4] Department of Conservation, Christchurch, NZ

Two experiments using rainbow trout were conducted to examine the combined effects of energy intake as manipulated by ration and pulp and paper mill effluent exposure over either one or two consecutive reproductive cycles. This study demonstrated that the level of energy intake affected the full range of measured parameters, from energy allocation to somatic growth and the gonads, steroid production, and hematology. Increasing ration level expectedly increased growth, condition, liver and gonad size. Sex steroid levels and hematological parameters were also positively influenced by increasing ration level in males and females. By far the most dramatic impact of reduced ration on reproduction was to substantially reduce the frequency of sexually maturing fish. The physiological effects of pulp and paper effluent exposure observed in these experiments were not consistent between the two experiments conducted herein, nor were they consistent with previously observed impacts in similar experiments with this effluent. Effluent exposure over one reproductive cycle did not impact physiological parameters in trout. However, when effluent exposure was maintained over two reproductive cycles, a new pattern of effluent response emerged, including increased condition factor in both sexes and increased sex steroids and reproductive investment and growth in males. The observed effects of effluent were not significantly modified by ration. The effects of ration were far more obvious and consistent when a longer exposure was employed. Thus, it appears to takes more than one full year for energy intake changes to be reflected in those particular physiological endpoints measured.

Experimental designs and assessment methods for evaluating the environmental effects of effluents. ***K. Somers[1], B. Keller[1] and C. L. Sarrazin-Delay[2] (PL)***
[1]Ontario Ministry of the Environment, Dorset, ON; [2]Laurentian University, Udbury, ON

A variety of experimental designs have been proposed for assessing the environmental effects of effluents. The Reference Condition Approach (RCA) is a relatively new experimental design that is gaining popularity. Typically, the RCA compares biological condition at an unknown test site with the average biological condition of a number of minimally impacted reference sites. The appropriate number of reference sites and the definition of minimally impacted conditions are often debated. Additionally, there are many ways to assess the magnitude and significance of the observed difference between a given test site and the reference sites. To date, very few studies have compared the results of different RCA-based assessment techniques. Similarly, the accuracy of different assessment methods is largely unknown. Using data collected as part of a collaborative northern Ontario RCA study of metal-mining effects, we compare assessment techniques with a combination of real and simulated data to gain a better understanding of the strengths and weaknesses of different assessment methods.

Potential of assessing invertebrates at the population level in monitoring programs. T. Arciszewski[1], K. Munkittrick[2] and K. A. Kidd[2] (PL)

[1]Canadian Rivers Institute, Saint John, NB; [2]University of New Brunswick Saint John, Saint John , NB

Community-level approaches are traditionally used when conducting bioassessments with benthic invertebrates. Several discharges enter the Saint John River at Edmundston, NB, and three previous cycles of monitoring have shown disagreements between the signals from fish population and invertebrate community data, and an inability to clearly differentiate the relative importance of the sewage and pulp mill outfalls. We examined the utility of invertebrate population endpoints to increase our resolution of the issue, and compared the life history characteristics of stonefly larvae to responses to similar endpoints in slimy sculpin *(Cottus cognatus)*, which are known to have a small home range. We sampled the larval stoneflies *Acroneuria* sp. and *Paragnetina* sp., and were able to externally discriminate males and females. Both the sculpin and the stoneflies showed eutrophication responses downstream of the sewage effluents, and there were consistent responses between three stonefly species, in both males and females. The stoneflies showed increased abundance, size, development, condition, and fecundity, whereas the sculpin showed increased abundance, size, growth, and condition. At this site, there was no detectable response to pulp mill effluent released downstream of the sewage outfalls, although invertebrate community responses showed a slight impairment at this site. Our findings support the investigation of invertebrate population assessments as an alternative method in environmental monitoring programs.

Wild blue mussels (Mytilus edulis) as a fish sentinel species for marine EEM studies. K. Connors[1], P. Orr[1] and C. Russel[1] (PL)

[1]Minnow Environmental Inc., Georgetown, ON

Wild blue mussels were used as sentinel fish species for two maritime pulp and paper mill EEM's in place of a conventional fin-fish survey. Two consecutive EEM cycles of data were collected at one of these sites. Wild mussels proved to be an effective sentinel species as they are sedentary, confirming persistent effluent exposure, relatively abundant and could be readily collected at both mill sites and reference areas. The use of wild blue mussels allowed for the same population health endpoints as used in a conventional fin-fish survey, unlike the caged bivalve in situ studies. Habitat differences between reference and exposure areas can confound the interpretation of results. However, mussels were found in both the intertidal or subtidal habitats, allowing a greater opportunity to sample from consistent habitat between areas. Lastly, the survey proved to be a cost-effective approach with minimal ecological impact (i.e. no fish bycatch). The merit of various measures will be evaluated analogous to traditional fin-fish endpoints. In addition, the results will be discussed in terms of potential mill-related effects, in relation to the benthic survey results, and with respect to which metrics may be appropriate for inclusion in future wild blue mussel studies.

Characterizing reference conditions in the South Nahanni River, NWT, Canada, a World Heritage Site and its sensitivity to mine developments at periphyton, benthic macroinvertebrate, and fish population-and community-levels of response. ***M. Bowman[1], P. Spencer[2], D. West[2] and M. D. Dubé[2] (PL)***
[1]Utah State University, Logan,UT; [2]University of Saskatchewan, Saskatoon, SK

Canada's north is changing at an unprecedented rate as a result of global warming and increased resource development in sectors such as oil and gas and mining. The goal of this study was to contribute to the understanding of variability in aquatic reference conditions of the North and to identify species or communities that are feasible and sensitive indicators of human disturbance. Sediment type and chemistry, water chemistry, and periphyton, benthic macroinvertebrate, and fish communities were characterized at 20 reference sites and at near-field and far-field sites downstream of two metal mines. Comparisons between the upstream, near-field, and far-field study design versus a reference-condition approach showed that for detecting biological effects, setting an appropriate ecological threshold was of greater consequence than the type of study design used. Biological indicators most sensitive to environmental change included benthic algal and invertebrate abundances and community structure that were consistent with mild eutrophication effects. Using a regional, multitrophic-level approach to monitor the effects of human disturbance improves inferential strength and broadens applicability.

Nutrients in the Upper Athabasca River: Monitoring to fit the need. ***M. Spafford[1] and P. McEachern[2] (PL)***
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The Athabasca basin is one of the largest drainages in Alberta, with an area of 155,000 square km. Resource development is the major land use activity and oil and gas development is extensive. The main users of water and sources of effluents discharged directly or indirectly to the Athabasca River in the upper basin are pulp mills, agriculture and municipalities. Eutrophication has been identified as the largest threat to ecosystem integrity in the upper basin. The Northern River Basins Study, provincial monitoring in the 1980s and 1990s, and the Environmental Effects Monitoring program (EEM) have all identified nutrients as the number one risk. Detailed monitoring under the Canadian Environmental Effects Monitoring (EEM) program has indicated mild eutrophication of localized zones downstream from pulp mills. However, the EEM only targets 10% of total nutrient loading (just pulp mills). While EEM provides a useful snapshot for a minor component of the problem, it is not a sound foundation for managing sustainable development at the basin scale. Alberta is developing a strategy to preserve and enhance water quality throughout the province based on in-stream targets that are condign with cumulative effects from all contributors to water quality and quantity impacts. The targets form the basis of management activities with goals ranging from anti-degradation to use protection (effects thresholds). The case study presented here occurred under the auspices

of the Athabasca Water Quality group created in 2005, and it provides a précis of: 1) the development of predictive tools to determine site-specific risks and environmental capacity, and 2) the proposed basin management framework that supports sustainable development and adaptive management.

National Investigation of Cause project on reduced gonad sizes in fish: Progressing towards causes and solutions. M. Hewitt[1], T. Kovacs[2], D. MacLatchy[3], P. Martel[2], M. McMaster[1], J. Parrott[1], G. Van Der Kraak[4] and M. van den Heuvel[5] (PL)

[1]Environment Canada, Burlington, ON; [2]Forest Products Innovation Paprican, Pointe Claire, QC; [3]Wilfrid Laurier University, Waterloo, ON; [4]University of Guelph, Guelph, ON; [5]University of Prince Edward Island, Charlotteoton, PE

The first three cycles of Environmental Effects Monitoring (EEM) in the pulp and paper sector show two consistent national response patterns in fish: responses related to nutrient enrichment, and responses described as metabolic disruption. Metabolic disruption is described by larger liver size, smaller gonad size and increased body weight. Effective and economical mitigating solutions for effects of mill effluents on fish reproduction have yet to be identified. In 2005 a team comprised of researchers from industry, government and academia was assembled and a five-activity plan to evaluate in-mill and end-of-pipe treatment options for removing substances affecting fish reproductive capacity from mill effluents was formulated. Research got underway in 2006 determining which laboratory tests will provide the most appropriate measures of gonad size reductions seen in wild fish. The results of the first mill effluent studied show that wild fish results can be reproduced with laboratory lifecycle experiments and that egg production in shorter-term tests show promise for investigating causes and solutions.

Overview of Cycle 4 pulp and paper Environmental Effects Monitoring studies. C. Tessier[1], R. Lowell[1], A. Willsie[1], G. Kaminski[1] and C. Gaudet[1] (PL)

[1]Environment Canada, Gatineau, QC

Enacted under the federal Fisheries Act, the 1992 Pulp and Paper Effluent Regulations (PPER) require mills that deposit effluents into Canadian waters to undertake an Environmental Effects Monitoring (EEM) study to monitor fish, fish habitat and the usability of fisheries resources. To date, four cycles of biological monitoring have been completed by mills across the country. National assessments of cycles 1 through 3 provided a good picture of the nature of the effects of Canadian pulp and paper mill effluents on receiving biota, the primary effects being 1) nutrient enrichment and 2) a reduction in fish gonad size. In cycle 4, mills that did not confirm effects conducted a standard survey. For mills that found repeatable, large effects in either the invertebrate or fish surveys, the cycle 4 EEM study attempted to determine the extent and magnitude of the effects or to investigate the cause of the effects. An overview of the studies conducted for cycle 4 of the pulp and paper EEM, as well as some preliminary results, are

presented. A broad outline of the approaches and methods employed to implement Investigation of Cause (IOC) work done for the first time in cycle 4 at 28 mills across Canada is also presented.

Smart Regulation for the pulp and paper industry in Canada: Three years later. G. Kaminski[1] (PL)

[1]Environment Canada, Gatineau, QC

During fifteen years of pulp and paper environmental effects monitoring (EEM) in the receiving environment, the EEM program has been reviewed following each cycle, culminating in a major multi-stakeholder "Smart Regulation" initiative in 2005. Based on this experience and the 2005 review, EEM has become a model for smart regulation and is undergoing significant scientific evolution to ensure that it is delivered efficiently and effectively. Two key initiatives have already started: the national Investigation of Cause (IOC) for fish gonads project and the IOC projects on eutrophication based on pronounced eutrophication guidelines developed by the National EEM Team. Industry is conducting studies that will lead to solutions for eutrophication. Several other issues raised during the Smart Regulation review, such as: 1) better use of critical effect sizes (CES), 2) expanding options for alternative fish surveys, 3) developing "triggers" based on existing sublethal toxicity tests and/or methods developed during the national IOC project for fish gonads initiative, and 4) solving the problem of significant statistical interactions between exposed and reference sites for fish surveys, will be evaluated by Environment Canada.

Canadian metal mining Environmental Effects Monitoring: Overview of program review recommendations. R. Prairie[1] and D. Gautron[2] (PL)

[1]Xstrata Zinc Canada, Saint-Laurent, QC; [2]Environment Canada, Gatineau,QC

Since the promulgation of the Metal Mining Effluent Regulations (MMER) under the Fisheries Act in 2002, all mines subject to the MMER are required to conduct an Environmental Effects Monitoring (EEM) program to evaluate the effects of metal mine effluents on fish, fish habitat, and the use of fisheries resources.

Based on Environment Canada's commitment to review the overall effectiveness of the program approximately three years after its commencement, a multi-stakeholder working group, the Metal Mining EEM Review Team, was formed in December 2005 to review all aspects of the EEM program and formulate recommendations to Environment Canada in order to improve its effectiveness, efficiency and scientific defensibility. The composition of the team included representatives from Environment Canada and other federal departments, industry, Aboriginal and environmental non-government organizations, and the Canadian Nuclear Safety Commission. The process took approximately 18 months to be completed.

Some 43 recommendations were made by the review team to Environment Canada. The recommendations dealt with program implementation, effluent characterization and water quality survey, sublethal toxicity, cross-cutting science issues, fish survey and benthic

invertebrate survey. The level of recommendations ranged widely, from various improvements to the EEM guidance and up to modifications to MMER requirements. The presentation will provide an overview of the review team's process and of the final recommendations made to Environment Canada.

National trends and regional site-specific application of sublethal toxicity data under the metal mining EEM program from the initial monitoring phase (2003 to 2005). L. Taylor[1], L. Van der Vliet[1] and R. Scroggins[1] (PL)

[1]Environment Canada, Ottawa, ON

Since 2002, metal mining operations in Canada have been required under the Metal Mining Effluent Regulations to conduct Environmental Effects Monitoring (EEM) studies. One component of the EEM program is the conducting of sublethal toxicity (SLT) tests on the principal effluent discharge. During the three-year Initial Monitoring phase, mines completed SLT testing using a battery of four tests at a frequency of twice per year. An analysis of this data has been completed by staff of EC's Biological Methods Division. In this presentation, some of the national trends of SLT data focusing on test sensitivity/responsiveness, differences between regions and differences between major mine categories will be covered. Examples of how the SLT data are being used on a regional and site-specific level will also be provided. Specific uses of the data include: 1) identifying differences in effluent variability across major mine categories within a region; 2) helping with Final Discharge Point (FDP) selection; 3) assessing changes in wastewater treatment at a site; and 4) assessing the relationship between SLT data and field results.

National assessment of potential toxicants based on the 2005 MMER-EEM sublethal toxicity and mine effluent chemistry data. B. Vigneault[1], S. Krack[1], and M. King[2] (PL)

[1]Natural Resources Canada, Ottawa, ON; [2]CANMET-Mining and Mineral Sciences Laboratories, Ottawa, ON.

Abstract

Sublethal toxicity and chemistry data are collected to monitor effluent quality as part of the Metal Mining Effluent Regulations – Environmental Effects Monitoring Program (MMER-EEM). To identify potential toxicants, correlation analyses were conducted on the chemistry and toxicity data collected at operating metal mines across Canada in 2005. The chemistry data were then compared to available toxicity values. As expected, only a few chemical parameters correlated with sublethal toxicity responses. No negative correlation was found for the growth inhibition of fathead minnows (*Pimephales promelas*) or *Lemna minor* based on dry weight. Correlations with hardness and cadmium were found for growth inhibition of *L. minor* based on the number of fronds. However, based on the effluent concentrations and reference toxicity data for *L. minor*, cadmium can be excluded as a potential toxicant. *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricorntum*) growth inhibition was negatively correlated with hardness,

cadmium and zinc, but only cadmium could be excluded as a potential toxicant. For *Ceriodaphnia dubia*, significant negative correlations were found with hardness, arsenic, lead and nickel. Based on available data, only hardness and nickel are likely potential toxicants for *C. dubia*. It is concluded that potential toxicants are zinc for *P. subcapitata*, nickel for *C. dubia*, and hardness for all test species except *P. promelas*.

Introduction

The Metal Mining Effluent Regulations (DFO, 2002) include the requirement for sublethal toxicity testing and the determination of additional chemical parameters in effluent as part of the Environmental Effects Monitoring Program. For the first three years of the MMER-EEM most mines have conducted four sublethal toxicity tests on a semi-annual basis: growth inhibition for the macrophyte *Lemna minor*, the algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricorntum*) and fathead minnow larvae *(Pimephales promelas)*, and reproduction inhibition for *Ceriodaphnia dubia*. The MMER-EEM also requires monitoring of aluminium, cadmium, iron, mercury, molybdenum, ammonia and nitrate in addition to deleterious substances (arsenic, copper, cyanide, lead, nickel, zinc, total suspended solids, radium-226 and pH) on the sample tested for sublethal toxicity.

Few studies have examined the potential relationships between effluent sublethal toxicity and chemistry. In 1997, a study on eight mine effluents was completed as part of the Aquatic Effects Technology Evaluation Program (AETE, 1997). Sublethal toxicity tests were conducted on *L. minor*, *P. subcapitata*, *C. dubia* and fathead minnows. Effluent chemistry measurements included dissolved solids (TDS), total suspended solids (TSS), ammonia, conductivity, alkalinity, pH, hardness, zinc and copper. Only four significant correlations were found: *P. subcapitata* versus TDS, conductivity and hardness and *L. minor* versus TSS. This lack of correlation was attributed to elevated detection limits for metals ($\geq 10\ \mu g \cdot L^{-1}$).

The objectives of the current study are to provide recommendations to improve MMER-EEM and orient research efforts on effluent toxicity and possible treatment methods. Using the MMER-EEM effluent chemistry and sublethal toxicity data collected in 2005, potential toxicants were identified based on correlation analysis and comparisons with reference toxicity values.

Material and Methods

The 2005 MMER-EEM effluent sublethal toxicity and chemistry data were reviewed. Minimal modifications were made to the electronic dataset—for example, uniformly reporting as recommended no significant inhibition or inhibition less than 25% as > 97% for *L. minor*. Some data for *L. minor* were also excluded since the actual endpoint was not specified—i.e., dry weight or fronds number. The sublethal toxicity and chemistry data were analyzed by Spearman rank order correlations (using SigmaStat version 3.5, Systat Software Inc. 2006) in order to identify significant negative relationships between the reported inhibition and chemical parameters.

For chemical parameters with negative correlation(s), toxicity tests were then conducted using MMER-EEM protocols for *L. minor* (Env. Can. 1999), *P. subcapitata* (Env. Can. 1992a) and *C. dubia* (Env. Can. 1992b). For *L. minor*, tests were conducted with two clones in order to identify potential differences in sensitivity (UTCC 490 and UTCC 492 from the University of Toronto Culture Collection). For *P. subcapitata*, additional testing for cadmium and zinc was also conducted with the second protocol recommended in the MMER-EEM (CEAEQ, 1997). For all tests, 25% inhibition concentrations, IC_{25}, were estimated based on measured concentrations using Comprehensive Environment Toxicity Information System (CETIS version 1.025B, Tidepool Scientific Software, 2004). For a given chemical parameter, toxicity unit, TU, were calculated for each effluent sample according to:

$$TU = \frac{[\,] \; (mg \cdot L^{-1})}{IC_{25} \; (mg \cdot L^{-1})}$$

where [ ] is the concentration of the chemical substances in the effluent. Chemical parameters for which TU were much less than 1 were excluded from the list of potential toxicants. For chemical parameters with TU ~ 1, it was assumed that they could contribute to the observed toxicity. Finally, chemical parameters with TU much greater than 1 were identified as potential toxicants.

Results

For the growth of fathead minnow larvae, the majority of samples tested had $IC_{25} > 100\%$ and no correlations were found between the IC_{25} and any of the chemical parameters. Elevated hardness was identified as a potential cause of toxicity for *L. minor*. Based on dry weight, 15 out of 72 samples had $IC_{25} > 97$ % and the largest inhibition had an IC_{25} of 10.4 %. No negative correlations were found between IC_{25} based on dry weight and any of the chemical parameters. Sensitivity was greater based on the fronds number, as 41 out of 72 samples had $IC_{25} > 97$ % and the largest inhibition had an IC_{25} of 1.7 %. Negative correlations were found between IC_{25} based on fronds number with cadmium ($P < 0.05$, $r = -0.31$) and hardness ($P < 0.05$, $r = -0.48$). The IC_{25} for cadmium was similar for the two clones, with IC_{25} of 18 (4 – 25) and 21 (n.a. - 44) $\mu g \cdot L^{-1}$ for UTCC 490 and UTCC 492 respectively. In comparison, the actual effluent concentrations were much lower and only 1 out of 54 samples had a TU for cadmium higher than 1, i.e. a TU of 1.5. Therefore, cadmium is not generally associated with the observed toxicity. Sensitivity to calcium was also similar for both clones, with IC_{25} of 50 (n.a. – 107) and 65 (14 - 174) $mg \cdot L^{-1}$ for UTCC 490 and UTCC 492 respectively. In contrast to cadmium, the pooled IC_{25} for hardness, 144 mg $CaCO_3 \cdot L^{-1}$ (Table 1), is well within the range observed for the effluents, 40 to 5550 mg $CaCO_3 \cdot L^{-1}$, and TU much higher than 1 were calculated (Figure 1). With the highest TU in the 30-40 range, hardness was identified as a potential toxicant for *L. minor* growth inhibition (based on fronds number).

Elevated hardness and zinc were identified as potential toxicants for *P. subcapitata.* For the algal test, 31 out of 72 samples had $IC_{25} > 100$ % and the largest inhibition was an

IC_{25} of 2 %. Negative correlations were found between IC_{25} and cadmium ($P < 0.05$, $r = -0.51$), zinc ($P < 0.05$, $r = -0.34$) and hardness ($P < 0.05$, $r = -0.24$). Sensitivities to cadmium and zinc were about 17 times higher using the CEAEQ (1997) protocol compared to the Env. Can. protocol (1992) with IC_{25} of 4.2 (0.8 – 9.4) and 71 (58 -77) $\mu g \cdot L^{-1}$ respectively for cadmium and IC_{25} of 2.4 (1.2 - 3.1) and 41 (38 - 44) $\mu g \cdot L^{-1}$ respectively for zinc. In 2005 however, all the tests were conducted using the Env. Can. protocol. For cadmium, the IC_{25} of 55 $\mu g \cdot L^{-1}$ (Table 1), was higher than the range of concentrations reported for the effluent, < 0.01 to 36 $\mu g \cdot L^{-1}$, and it was excluded as a potential toxicant for *P. subcapitata*. In contrast, the IC25 for zinc, 57 $\mu g \cdot L^{-1}$ (Table 1) was well within the range of concentrations for the effluents, < 1 – 320 $\mu g \cdot L^{-1}$, and TU up to 5.5 were calculated (Figure 2). Therefore, zinc was identified as a potential toxicant for *P. subcapitata* growth inhibitions.

Lastly, for *C. dubia*, both hardness and nickel were identified as potential causes for the observed toxicity. For the reproduction test, 53 out of 82 samples had $IC_{25} > 100$ % and the largest inhibition had an IC_{25} of 0.8 %. Negative correlations were found between IC_{25} and arsenic ($P < 0.05$, $r = -25$), lead ($P < 0.05$, $r = -0.25$), nickel ($P < 0.05$, $r = -0.35$) and hardness ($P < 0.05$, $r = -0.34$). Arsenic was tested as arsenate, the dominant arsenic species in mine effluents (Williams, 2001). The IC_{25} for arsenic was 1.2 $mg \cdot L^{-1}$ (Table 1), well above the reported concentrations, < 0.0002 – 0.38 $mg \cdot L^{-1}$. Accordingly, it was concluded that arsenic is probably unrelated to the observed *C. dubia* reproduction inhibition. The IC_{25} obtained for lead, 31 $\mu g \cdot L^{-1}$ (Table 1) is however closer to the range of effluent concentrations, < 1 – 30 $\mu g \cdot L^{-1}$. With 12 out of 71 samples having a TU close to 1, lead was identified as a toxicant that might contribute to the observed toxicity. Lastly, the IC_{25} for hardness was 1246 $CaCO_3 \cdot L^{-1}$ (Table 1), which is within the range of reported hardness for the effluents, 40 to 5550 mg $CaCO_3 \cdot L^{-1}$. Hardness was identified as a potential toxicant, with 19 out of 83 samples with TU > 1 and up to ~4 (Figure 1).

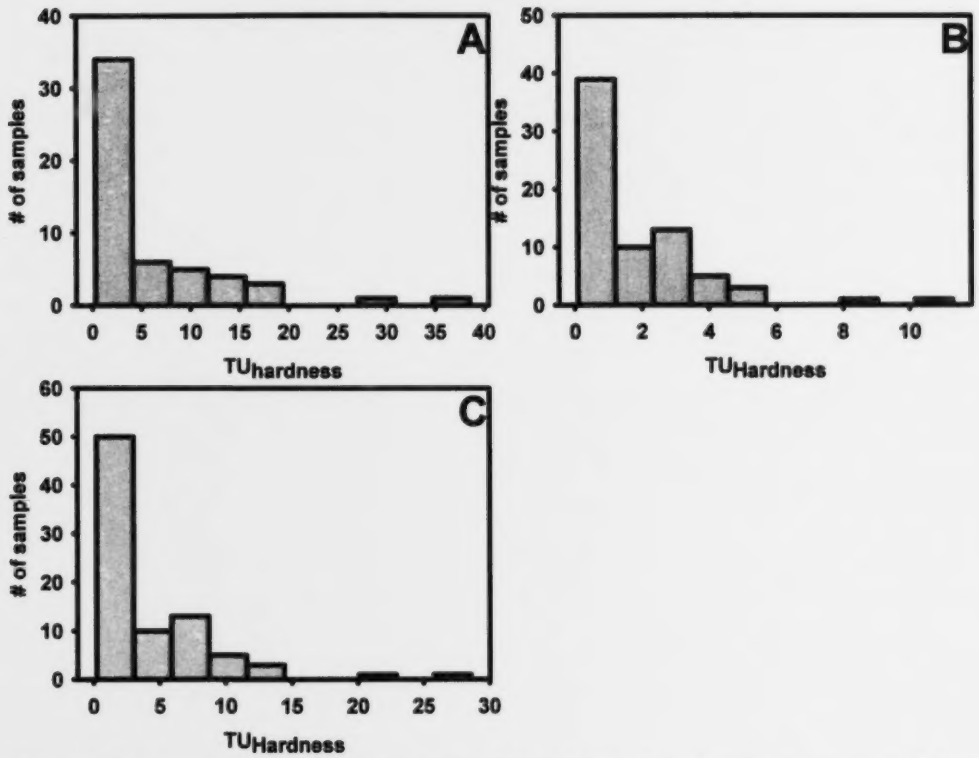

Figure 1 Calcium and magnesium as potential toxicants for: *Lemna minor* growth based on number of fronds, (A), *Pseudokirchneriella subcapitata* growth (B), and *Ceriodaphnia dubia* reproduction (C). The toxic unit for hardness, $TU_{hardness}$, was calculated by dividing the effluent concentrations by the measured IC_{25} (Table 1).

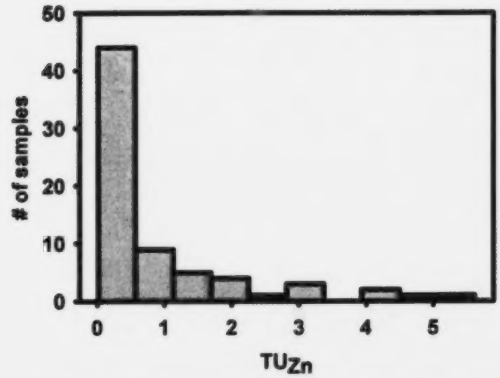

Figure 2 Zinc as a potential toxicant for *Pseudokirchneriella subcapitata* growth. The toxic unit for zinc, TU_{Zn}, was calculated by dividing the effluent concentrations by the measured IC_{25} (Table 1).

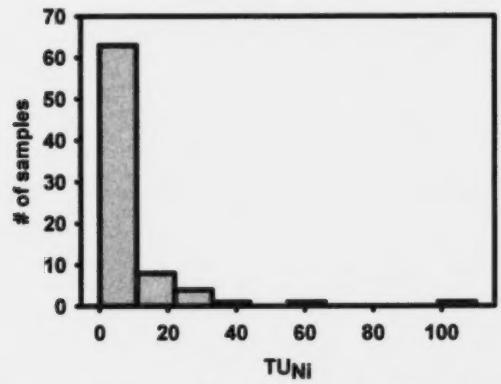

Figure 3 Nickel as a potential toxicant for *Ceriodaphnia dubia* reproduction. The toxic unit for nickel, TU_{Ni}, was calculated by dividing the effluent concentrations by the measured IC_{25} (Table 1).

Table 1 Measured 25% inhibition concentrations, IC_{25}, used to calculate toxic units for the growth of *Lemna minor*, *Pseudokirchneriella subcapitata* (Env. Can. protocol) and *Ceriodaphnia dubia*. IC_{25} are mean ± standard deviation if $n > 1$.

Test species	Chemical	IC_{25} $mg \cdot L^{-1}$	n	Note
L. minor (fronds)	Cadmium	0.023 ± 0.010	4	Tested as $CdSO_4$
L. minor (fronds)	Hardness	144	1	Tested as $CaSO_4$
P. subcapitata	Cadmium	0.055 ± 0.013	3	Tested as $CdSO_4$
P. subcapitata	Zinc	0.057 ± 0.018	5	Tested as $ZnSO_4$
P. subcapitata	Hardness	492	1	Estimation only
C. dubia	Arsenic	1.2	1	Tested as Na_2HAsO_4
C. dubia	Lead	0.031	1	Tested as $PbCl_2$
C. dubia	Hardness	1246		Tested as $CaSO_4$
C. dubia	Nickel	0.007 ± 0.006	3	Tested as $NiSO_4$

Conclusion

The conducted toxicity tests provided information regarding the MMER-EEM program. No differences in sensitivity for calcium or cadmium were observed for the two clones currently used for *L. minor* toxicity testing under the MMER-EEM. Our data therefore suggest that the individual analysis of the two clones of *L. minor* in the national dataset may be unnecessary. However, large differences in sensitivity to cadmium and zinc were observed for the two protocols recommended in the MMER-EEM for *P. subcapitata*. Accordingly, data obtained with the two protocols should be analyzed separately. Furthermore, it should also be noted that total metal concentration is a poor predictor of metal toxicity and the actual metal toxicity is likely overestimated based on total metal concentrations (e.g. complexation by dissolved organic carbon, DOC). It should also be noted that direct toxicity and effect on metal toxicity can be different for calcium and for magnesium. In addition, the limited chemical characterization of the effluent limits the extent of the analysis since potentially toxic contaminants may not be monitored. Therefore, other effluent chemistry parameters such as DOC, calcium and magnesium would be useful in future efforts to link effluent chemistry with environmental effects. Nevertheless, potential toxicants were identified for *L. minor*, *P. subcapitata* and *C. dubia* from the existing data. Based on correlation analysis and comparisons with reference toxicity values, three potential toxicants were identified: hardness (for *L. minor*, *P. subcapitata* and *C. dubia*), zinc (for *P. subcapitata*) and nickel (for *C. dubia*).

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***Weight of evidence approach for developing a site-specific water quality objective for copper at a proposed mine in northern British Columbia.* C. Totman[1], T. Watson[1], H. McLeod[2] and H. Bent[3].**

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Abstract

Effluent discharges from a previously considered mine in northern BC were projected to contain a number of constituents, with copper being an element of principal concern. Establishment of preliminary water quality objectives (PWQOs) in BC includes a number of methods such as adoption of generic water guidelines and modification of criteria to derive site-specific objectives. A recalculation procedure was adopted whereby a toxicological data set for copper was generated, reflective of site water quality and species resident to the site. This was necessary since available toxicological data did not adequately reflect species composition at the site. The toxicity data set was refined based

on information published by the US Environmental Protection Agency (USEPA) and supplemented by data from relevant Canadian jurisdictions. The sensitive-species distribution (SSD) approach and the biotic ligand model (BLM) were also applied to the database to develop copper PWQOs through a weight of evidence approach. The SSD and weight of evidence approach had not yet been used in BC and the provincial Ministry of Environment indicated it was more comfortable with SSD rather than BLM. Resulting copper criteria yielded a 30-day average PWQO of 7.5 $\mu g \cdot L^{-1}$ and a maximum objective of 10.5 $\mu g \cdot L^{-1}$. The results are discussed relative to other PWQOs and highlight the utility of the weight of evidence approach.

Introduction

The development of site-specific preliminary water quality objectives (PWQOs) for total copper was undertaken as part of the Environmental Impact Assessement (EIA) completed for Northgate Minerals Corporation's proposed Kemess Mine Expansion – Kemess North Copper/Gold Project (the Project). The EIA was pursuant to, and was reviewed under, a joint panel review process subject to the requirements under the *BC Environmental Assessment Act* and the *Canadian Environmental Assessment Act.* Although the mine will not be developed, the work conducted has relevance to other mines and project activities where the development of site-specific objectives is appropriate.

At a meeting with provincial agencies, the different procedures to develop WQOs were reviewed including background concentration, resident species, water effect ratio (WER), and recalculation procedure. The latter was identified as the preferred methodology as it is recommended for deriving objectives at sites with atypical assemblages of aquatic organisms (MacDonald, 1997). During the meeting the provincial agencies also confirmed toxicity data could be compiled from existing toxicity databases and sensitive species distribution (SSD) and, further, that the biotic ligand model (BLM) could be used as part of complementary procedures to develop PWQOs for copper. The aggregate considerations of these various methods comprised the "weight-of-evidence" approach adopted for copper.

Methods

The procedures used to develop copper PWQOs (recalculation and application of SSD, recalculation and application of an acute to chronic ratio (ACR) to the lowest acute toxicity value and BLM) are discussed below. It should be noted the first two methods were site-specific while BLM was not site-specific in that it included toxicity values for species not present on site.

Water quality models developed by Klohn Crippen Berger (KCB 2006) predicted water quality of the receiving waters downstream of the Project under various scenarios and were used to compare with the final PWQOs.

Recalculation procedure

Toxicity data sets used to derive the BC and Canadian maximum and average water quality guidelines for copper for the protection of aquatic life were compiled and supplemented with toxicity data obtained from Alberta Environment and the USEPA (AE 1996; USEPA 2003, 2007). Field studies conducted in the project area (Hatfield 2004) were used to identify species present in the receiving environment. Toxicity values for species not present in the receiving environment were removed from the data set as per the recommended methodology (MacDonald 1997).

Data normalization

A relationship between copper acute toxicity and water hardness (as $CaCO_3$) has been identified by the USEPA (2003). To derive PWQOs representative of the predicted receiving water quality for the various modeled scenarios, acute toxicity values for copper were adjusted to the predicted hardness using the equation provided in Stephan *et al.* (1985) and the USEPA (2003) slope value, except where it was not possible because hardness was not reported. The relationship between chronic toxicity values and corresponding water hardness was equivocal (AE, 1996; USEPA, 2003) and consequently chronic toxicity values were not normalized.

Sensitive Species Distribution (SSD)

SSD can be defined as a probabilistic model for the variation of the sensitivity of biological species for a certain compound or a set of compounds (Aldenberg *et al.*, 2002). SSD has been used to derive ambient water quality criteria in the US and in the Netherlands by combining acute or chronic toxicity values for several species to assess the concentration of a chemical below which an acceptable small percentile (p) of the species is affected (the Hazardous Concentration for p% of the species or HCp) (Aldenberg *et al.*, 2002). The E_TX 2.0 model (Van Vlaardingen *et al.*, 2004) was used to calculate the HCp for the site-specific species. The level of protection was selected, in consultation with agencies, at 95 % of the species. A HC that will exceed no more than 5% of the species acute or chronic toxicity values was calculated (HC_5). HC_5 were calculated using the revised acute and chronic data sets [no observed effect level (NOEL), lowest observed effect level (LOEL) and chronic value]. Chronic values were obtained by calculating the geometric mean of a given species NOEL and LOEL. Chronic values are considered to be representative of a threshold effect by the USEPA (USEPA, 2007). When more than one acute or chronic value was shown for a given species the geometric mean was used.

Biotic Ligand Model

BLM has been recently used by the USEPA to develop the ambient freshwater copper guideline (USEPA 2003 and 2007). The BLM is a "bioavailability" model explicitly accounting for individual water quality variables modifying metal toxicity which are not factored into the hardness-dependent criteria relationship (USEPA, 2003). The BLM (Model 2.2.0) used by the USEPA (2003 and 2007) was applied to obtain PWQOs for the predicted water quality in the receiving environment (Hinckley Dan, Pers. Comm., 207).

The model used pre-selected toxicity inputs based on the concentration that causes mortality in 50% of the organisms tested (LC_{50s}) from 22 fish species, 15 invertebrate species and one amphibian. Water quality parameter inputs for the model consisted of water quality predicted by KCB (2006) for the receiving environment. The model outputs consisted of a final acute value (FAV) for dissolved copper. The FAV was then divided by the modeled ACR to obtain final chronic values.

Results and discussion

Results of the detailed water quality modeling completed by KCB (2006) indicated total copper in receiving water would range from 0.0041 to 0.011 $mg \cdot L^{-1}$ and hardness would range from 89 to 145 mg $CaCO3 \cdot L^{-1}$.

Site-specific fish, invertebrates and algae included in the revised data sets are shown in Table 1 with the lowest identified acute and chronic toxicity values shown in Table 2.

Table 1. Species included in the revised site-specific data sets

Fish species - family:	Invertebrates - order:	Alga - phylum:
Acute data set		
Rainbow trout (Salmonidae)	Plecoptera	Bacillariophyta
Bull trout (Salmonidae)	Diptera	Chlorophyta
Cutthroat trout (Salmonidae)	Oligochaeta	
Coho salmon (Salmonidae)	Gastropoda	
Chinook salmon (Salmonidae)		
Sockeye salmon (Salmonidae)		
Pink salmon (Salmonidae)		
Chronic data set		
Rainbow trout (Salmonidae)	Trichoptera	Chlorophyta
Bull trout (Salmonidae)	Diptera	
Brook trout (Salmonidae)	Gastropoda	
Brown trout (Salmonidae)		
Lake trout (Salmonidae)		
Coho salmon (Salmonidae)		
Chinook salmon (Salmonidae)		
Mountain whitefish (Salmonidae)		
Sculpin (Cottidae)		

Table 2. Lowest copper acute and chronic toxicity values for the revised toxicity data sets

	Toxicity Values ($\mu g \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$ $CaCO_3$)	Species	References
LC_{50}	2.8	9.2	rainbow trout	USEPA, 2007
LOEL	4.6	25	rainbow trout	AE, 1996

Safety Factor Consideration

The level of the safety factor applied to the toxicity values was refined rather than using the default value of 0.1. NOEL and LOEL data from USEPA (2003, 2007) and Alberta (1996) were reviewed to obtain NOEL/LOEL ratios for copper. Data were available for eight fish species and nine invertebrate species. NOEL/LOEL ratios ranged from 0.31 to 0.67 with an average of 0.53 (n=32). Based on these ratios and on the fact that the environmental fate and toxicity of copper to aquatic organisms is adequately understood, a safety factor of 0.5 was derived for copper. Agreement on the use of the 0.5 safety factor was reached with the regulatory agencies.

Sensitive species distribution (SSD)

The species for which acute or chronic toxicity data were used to obtain SSD are shown in Table 3. When acute toxicity data were used, an ACR was applied to the resulting acute HC_5 to obtain the chronic HC_5. This is consistent with the methodology used by the USEPA and Alberta Environment (AE 1996, USEPA 2003) to derive chronic criteria based on acute values when the chronic database is limited. Available geometric mean ACRs for site-specific species were as follows (USEPA 2003, USEPA 2007):

- 2.88 for rainbow trout;
- 5.594 for chinook salmon; and,
- 191.60 for the snail *Campeloma decisum*.

A final ACR of 4.01 was calculated based on the geometric mean of the rainbow trout and chinook salmon using the USEPA data. The snail ACR was not included as per the methodology provided by Stephan *et al.* (1985) as selected ACRs should be within a factor of 10. The resulting HC_5 for site-specific species based on acute toxicity values are shown in Table 4 and HC_5 based chronic toxicity values are shown in Table 5.

Recalculation and application of an acute to chronic ratio (ACR) and safety factor to the lowest acute toxicity value.

PWQOs ranging from 4.4 to 8 $\mu g \cdot L^{-1}$ for copper were obtained based on the lowest LC_{50} for rainbow trout normalized to predicted water hardness using the USEPA slope value (0.9584) (Table 6). The lowest adjusted LC_{50s} were divided by an ACR of 2.88 (for rainbow trout, USEPA, 2003) and a safety factor of 0.5 was subsequently applied to obtain PWQOs for the various modeled cases (Table 6).

Biotic Ligand ModelThe BLM final acute value (FAV) for dissolved copper ranged from 18.86 to 24.027 $\mu g \cdot L^{-1}$. The FAV were then divided by the model provided ACR of 3.23 to obtain the chronic values which ranged from 5.599 to 7.439 $\mu g \cdot L^{-1}$ (Hinkley D., Pers. Comm. 2007) (Table 7).

Summary of Copper PWQOs

The PWQOs obtained as part of the weight-of-evidence approach used for copper are summarized in Table 8 and range from 4.4 to 10.6 $\mu g \cdot L^{-1}$. The proposed PWQOs are in the lower range of PWQOs developed for copper as part of other selected projects (4.6 to 74 $\mu g \cdot L^{-1}$) (Table 9).

Table 3. Species for which acute or chronic toxicity data were used to obtain SSD

Common name	Scientific name	Acute SSD	Chronic SSD
Rainbow trout	Oncorhynchus mykiss	Yes	Yes
Brown trout	Salmo trutta	No	Yes
Brook trout	Salmo fontinalis	No	Yes
Bull trout	Salvelinus confluentus	Yes	No
Coho salmon	Onchorhynchus kisutch	Yes	No
Chinook salmon	Onchorhynchus tshawytscha	Yes	No
Pink salmon	Oncorhynchus gorbuscha	Yes	Yes
Cutthroat	Oncorhynchus clarkia	Yes	No
Sockeye salmon	Onchorhynchus nerka	Yes	Yes
Worm	Lumbriculus variegatus	Yes	Yes
Worm	Limnodrilus hoffmeisteri	Yes	Yes
Snail	Amicola sp.	Yes	No
Snail	Campelona decisum	No	Yes
Snail	Juga plicifera	Yes	No
Snail	Lithoglyphos virens	Yes	No
Snail	Physa integra	Yes	Yes
Midge	Chironomus decorus	Yes	Yes
Green alga	Selenastrum capricomutum	Yes	Yes

Table 4. HC_5 Obtained using a SSD approach with the acute toxicity data set adjusted to the predicted hardness values

Model Scenario	Predicted hardness ($mg \cdot L^{-1}$)	Predicted Cu ($mg \cdot L^{-1}$)	Acute HC_5 ($mg \cdot L^{-1}$)	*Chronic HC_5 ($mg \cdot L^{-1}$)
base case- pre pit release	89	0.0041	0.0213	0.0053
base case- post pit release	99	0.0042	0.0236	0.0059
upper bound - pre pit release	120	0.010	0.0284	0.0071
upper bound - post pit release	180	0.010	0.0418	0.010
7Q10 - pre pit release	139	0.011	0.0327	0.0082
7Q10 –post pit release	145	0.011	0.034	0.0085

*Chronic HC_5 = Acute HC_5 / 4.01

Table 5. HC_5 Obtained using a SSD approach with the chronic toxicity data sets

	Chronic HC5 ($mg \cdot L^{-1}$)	
NOEL	0.0057	
LOEL	0.0106	
Chronic value	0.0075	
Predicted Cu levels		0.0041-0.011

Table 6. PWQOs obtained based on the acute toxicity data set adjusted to the predicted hardness values using the ACR method

Model Scenarios	Predicted hardness ($mg \cdot L^{-1}$)	Predicted copper ($mg \cdot L^{-1}$)	Lowest $L_{50}C$ ($\mu g \cdot L^{-1}$) normalized to the predicted hardness	Resulting PWQOs ($mg \cdot L^{-1}$)
base case- pre pit release	89	0.0041	24.55 (rainbow trout)	0.0044
base case- post pit release	99	0.0042	26.81 (rainbow trout)	0.0046
upper bound - pre pit release	120	0.010	32.24 (rainbow trout)	0.0056
upper bound - post pit release	180	0.010	47.55 (rainbow trout)	0.008
7Q10 - pre pit release	139	0.011	37.12 (rainbow trout)	0.0064
7Q10 - post pit release	145	0.011	38.65 (rainbow trout)	0.0067

Table 7. BLM Results

Model Scenarios	Final acute value ($\mu g \cdot L^{-1}$)	Chronic value ($\mu g \cdot L^{-1}$)
upper bound - initial	23.499	7.275
upper bound - pre pit release	19.499	6.156
upper bound - post pit release	18.861	5.599
7Q10 – initial release	24.0267	7.439
7Q10 - pre pit release	20.125	6.231
7Q10–post pit release	20.519	6.353

Table 8. PWQOs obtained as part of the weight-of-evidence approach for copper

Methods	PWQOs ($\mu g \cdot L^{-1}$)
SSD (acute HC5/ACR)	5.3-10
SSD (chronic HC5)	5.7-10.6
Recalculation [(Hardness Adjusted lowest LC50/ACR)*SF]	4.4-8.0
BLM (dissolved)	5.6-7.5

Table 9. Examples of PWQOs for Copper

Location	Method	WQO ($\mu g \cdot L^{-1}$)	References
Oyster River, BC	Background Procedure	10 (average total)	Nagpal, 1990
Tsolum River, BC	Based on site-specific studies including background levels, organic complexing capacity, dilution, *in situ* acute and chronic toxicity	7 (dissolved average) 11 (dissolved max.)	Deniseger and Pommen, 1995
Boise River, Idaho	WER (*Ceriodaphnia dubia* and fathead minnow; 2.578) at a hardness of 50 $mg \cdot L^{-1}$	16 (dissolved average) 23 (dissolved max.)	CH2MHILL, 2002
Doris North Project, Yukon	BLM (based on background water quality)	4.6 (dissolved max.)	EVS, 2004
East Lake, ON	WER (Cerodaphnia dubia; 14.9) at a hardness of 60 $mg \cdot L^{-1}$	74 (acute) 34 (chronic)	Placer Dome

Conclusions

The main conclusions drawn with respect to developing PWQOs were: The weight-of-evidence approach has provided confidence in developing and recommending site-specific objectives where adjustment to toxicity data sets and species inclusion are necessary.

The methods used as part of the weight-of-evidence approach yielded similar PWQOs which facilitated reaching consensus with regulatory agencies. The weight-of-evidence approach led to the establishment of PWQOs for copper, as follows:

- A 30-day average objective of 7.5 $\mu g \cdot L^{-1}$
- A maximum objective of 10.5 $\mu g \cdot L^{-1}$.

A new version of the BLM is in development and should allow modification of toxicity values to reflect site-specific species. The model could be used to refine the values obtained with the generic BLM and it is expected these values will be similar or higher.

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Assessing the Impacts of Quebec Mining Effluents on Freshwater Benthic Invertebrates – EEM Phase 1. É. Lacroix[1], I. Matteau[1] and R. Chabot[1]
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Introduction
Under the Canadian Metal Mining Effluent Regulations (MMER), metal mines discharging into the aquatic environment are required to conduct environmental effects monitoring (EEM) studies. As part of the EEM Program, biological monitoring studies are performed to evaluate the effects of mining effluents on fish, fish habitat, and the use of fisheries resources.
We present a summary of phase 1 benthic invertebrate community surveys performed at 19 mines in Quebec. Eleven interpretive reports presenting the survey results were submitted in 2005 and eight others in 2006.

Methods
For all the mines, the control-impact approach (comparison between reference and exposure areas) was used.

- Benthos sampling was conducted over a few days between September 8 and November 3, 2004, and between August 20 and October 20, 2005.
- Receiving environments were composed of streams (10), lakes (5) and rivers (4), classified as either lotic (6) or lentic (13) waters, depending on flow.
- Sampling was done using either a standard Ponar grab (0.052 m^2), a small Ponar grab (0.023 m^2), a Hess sampler (0.1 m^2) or an Ekman grab (0.0529 m^2).
- Nine of the 19 mines identified benthic invertebrates to the family level, whereas the remaining ten mines achieved a lower level of identification.
- The determination of effects was based on significant statistical differences in abundance, taxonomic richness, Simpson's equitability index or Simpson's diversity index, and Bray-Curtis dissimilarity index between the exposure and reference areas. To determine response type, we referred to the critical effect size used in the EEM program for the pulp and paper sector: two standard deviations (SD) of the reference area mean (Lowell et al., 2007). A critical effect size for the mining sector has yet to be determined. Thus, the use of two reference SDs is shown here for information purposes only. Correspondence analyses and ETP/C (number of Ephemeroptera, Tricoptera and Plecoptera on number of Chironomidae) were also carried out at some sites as complementary data.

Table 1 Overview of the 19 Quebec mines that submitted their phase 1 EEM reports in 2005–2006

Mining Group	Site Name	Ore	Type of operations	Effluent	Effluent concentration in the exposed area
Base Metals	Bouchard-Hébert	Zinc, Copper	Underground	Combined mine water & tailings area effluent	≥ 77 %
	Copper Rand	Copper	Underground	Combined mine water & tailings area effluent	56-94 %
	Louvicourt	Copper, Zinc	Underground	Tailings area effluent	72%
	Matagami	Zinc, Copper	Concentrator	Tailings area effluent	17%
	Raglan	Nickel, Copper	Underground & open pit	Tailing stack water combined with mine water and sewage water & 2 mine water effluents	12%
Precious Metals	Beaufor	Gold	Underground	Mine water	Unknown
	Camflo	Gold	Concentrator	Tailings area effluent	Unknown
	Doyon	Gold	Underground	Combined mine water, tailings area water and waste rock piles seepage and runoff	3-29 %
	Géant Dormant	Gold	Underground	Tailings area effluent and mine water effluent	26-100 %
	Joe Mann	Gold	Underground	Mine water	26-100 %
	Kiena	Gold	Underground	Tailings area effluent	2-20 %
	Laronde	Gold	Underground	Combined mine water, tailings area water and waste rock piles seepage and runoff	86-100 %
	Mouska	Gold	Underground	Mine water	5-9 %
	Principale	Gold	Concentrator	Tailings area effluent	83-99 %
	Sigma	Gold	Open pit	Tailings area effluent	54%
	Troilus	Gold	Open pit	Tailings area effluent and Mine water effluent	30%
Others	Mont-Wright	Iron	Open pit	Tailings area efffluent and and waste rock piles seepage and runoff effluent	62%
	Niobec	Niobium	Underground	Combined mine water and tailings area eflfuent	76%
	Tio	Titanium, Iron	Open pit	Mine water	4%

Results

Data from four of the 19 studies were inconclusive due to differences in the characteristics of the habitats at the exposure and reference areas. The results presented below are therefore taken from the remaining 15 studies.

Of these 15 studies, ten still showed some variability between the exposure and reference area habitats. Other confounding factors include historical heavy-metal contamination at one site, mine site exfiltrations into the reference area at another, and a possible case of agricultural pollution.

A first look at the effects on benthic communities

All mines showed at least one effect, as revealed by the core endpoints (Figure 1). Five mines out of 15 presented three effects.

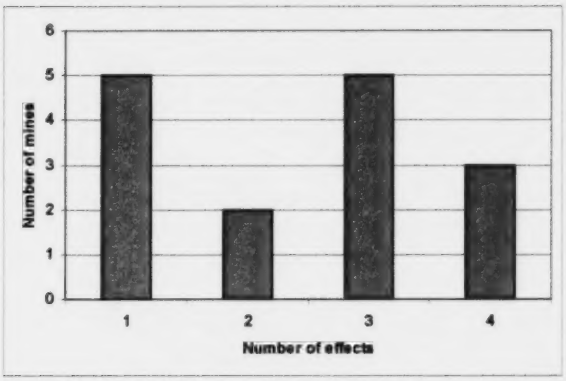

Figure 1 Number of effects revealed by the 15 benthic invertebrate surveys

All mines showed an effect on the Bray-Curtis (B-C) index (Table 2), meaning the communities in the two areas are different. Due to the method of calculation, Bray-Curtis differences are always positive. The mean magnitude of effect is four times the standard deviation (SD) from the reference area, which is higher than the critical effect size used in the EEM program for the pulp and paper sector.

- Eight of 15 mines showed an effect on benthic density. These effects were equally distributed between an increase and a decrease in benthic density.

Preliminary trends in the type of response

At three mine sites, inhibitory effects were observed (decrease in density and/or richness of more than two reference SDs). Another mine showed a trend toward an inhibitory effect (decrease in both density and richness of about 1 reference SD). Thus, these mining effluents seemed to be toxic for the benthic invertebrates in the receiving environment.

- The inhibitory effect concurred with the presence of nickel in sediments at one of these sites at levels exceeding the Toxic Effect Threshold of 61 mg/kg Ni under the sediment-quality criteria of SLC and MENVIQ (1992).

Table 2 Summary of results of benthic community univariate descriptors

	Number of mines					
	Effect	Relative proportion (%)	Increasing effect	Mean magnitude of effect (SD)	Decreasing effect	Mean magnitude of effect (SD)
Density (n=15)	8	53	4	7,3	4	2,0
Richness (n=15)	7	47	2	2,6	5	3,0
Diversity (n=15)	6	40	2	3,1	4	1,9
Evenness (n=5)	0	0	0	---	0	---
Bray-Curtis Index (n=15)	15	100	15	4,0	0	---

For three mine effluents, effects on the receiving environments were of a stimulatory nature (increase in density and/or richness of more than two reference SDs). Consequently, these mining effluents seem to cause an enrichment (or eutrophication) of the receiving environment.

- All three mines contained nitrogen compounds (nitrates and/or ammonia) in their effluent and showed high concentrations of those nutrients in the receiving water. For two of them, the difference between exposure and reference areas was ten times as high. These mining effluent discharges are thus a source of nutrients that cause enrichment of the receiving environment, as shown by the benthic surveys.

One other mine showed effects that could be associated either with a pronounced enrichment effect or a toxic effect (increase in density and decrease in richness of more than two reference SDs).
These results will be confirmed in the next round of monitoring.

Multivariate analyses
Twelve mines used multivariate tools such as correspondence analysis, thus confirming the difference between reference and exposed benthic invertebrate communities at their sites. As shown in Figure 2, the dominant invertebrate group was the Chironomidae family (class Insecta, order Diptera) for eight of 15 (53%) mines.
At sites where only family level was identified, more precise identification could be needed in subsequent studies to better understand the environmental effects. This might be the case at sites where chironomids are predominant, since these organisms present a wide range of pollution tolerance levels.

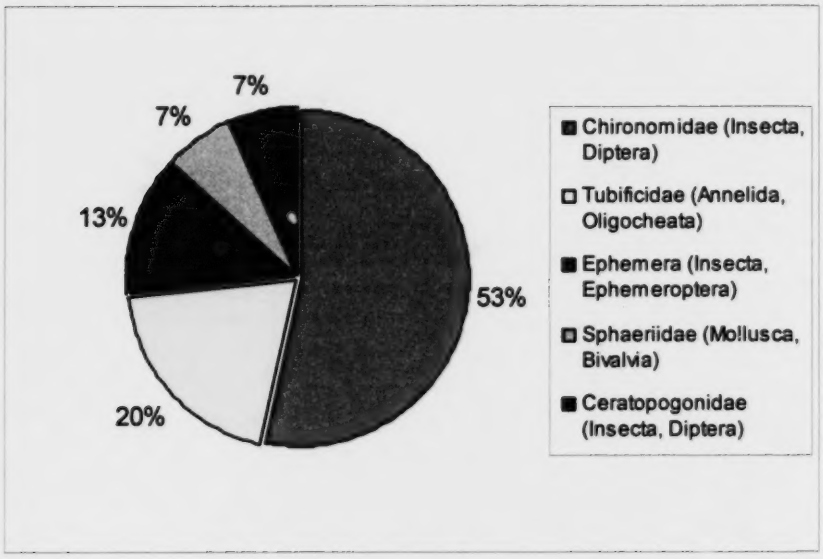

Figure 2 Dominant invertebrate groups of the 15 Quebec benthic surveys

Influence of effluent dilution

The four mines for which benthos were surveyed in an area exposed to an effluent concentration higher than 70% showed at least three effects, as revealed by the univariate descriptors. All four mines showed an inhibitory type of response or a trend toward an inhibitory effect.

Conclusion

First round of data highlights:

- Major differences in habitats were encountered between the exposure and reference areas for four of the mines surveyed. These differences made it impossible to conclude on the environmental effects of their effluents. Variability in habitat characteristics was also observed at some other mines.
- All mines showed an effect on the Bray-Curtis index; three out of 15 mines presented an effect on the four core endpoints.
- There were not enough mines per mining group to be able to determine the type of effect by type of ore produced.
- Inhibitory effects were observed for three mines; stimulatory effects were found for three others.
- By comparison, the national assessment report of phase 1 showed that effects on benthic invertebrates tended to be more inhibitory than stimulatory, although both kinds of effects occurred.
- Further rounds of data collection will measure the constancy or variability of these response patterns over time.

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Acknowledgements
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Summary of results from the first Environmental Effects Monitoring (EEM) benthic studies at Ontario mines and comparison with effluent sublethal toxicity. W. Plant[1] and N. Ali[1] (PO)

[1]Environment Canada, Downsview, ON

The Metal Mining Effluent Regulations require metal mines to conduct benthic invertebrate surveys in effluent receiving waters and sublethal toxicity tests on final effluent. Metal mines are required to report four standard endpoints for the benthic survey: total benthic invertebrate density, taxa richness, Simpson's Diversity and the Bray-Curtis Index. Four sublethal toxicity tests are required using standard methods referenced in the regulations, *Pimephales promelas*, *Ceriodaphnia dubia, Lemna minor* and *Pseudokirchneriella subcapitata*. The following poster discusses the benthic invertebrate community survey results for twenty Ontario metal mines and examines the relationship between the benthic data and effluent sublethal toxicity results using a qualitative lab-to-field rating scheme. The most common effects observed at Ontario mines were: higher total benthic invertebrate densities, lower taxa richness, lower Simpson's Evenness and higher Bray-Curtis values. Seventy-three percent of the lab-to-field relationships were determined to be either strong or moderate between the benthic invertebrate community results and the sublethal toxicity results for *C. dubia* reproduction.

The implications of variability in seasonal reproductive profiles of northern redbelly dace (Phoxinus eos) for designing Environmental Effects Monitoring programs. L. Carroll[1] and K. Munkittrick[1] (PO)

[1]University of New Brunswick Saint John, Saint John, NB

Several small-bodied cyprinid fishes have proven to be well suited for use as sentinel species in environmental effects monitoring programs. However, insufficient information on life history characteristics such as reproductive strategy, mobility, longevity, and food habitats for a number of species challenges the design of studies and restricts the interpretability. Several cyprinid species spawn multiple times throughout the summer, and seasonal variability in the relationships between gonad and body weights can influence the detection of differences in reproductive performance. Past studies have established that Northern Redbelly Dace *(Phoxinus eos)* exhibit high variability in gonad

weight: body weight relationships during the spawning season. The objective of this study was to follow seasonal changes in condition, liversomatic index, gonadosomatic index and ovarian histology to examine the reasons for the gonad development variability among female redbelly dace. Fish were collected monthly from two lakes within Rockwood Park, New Brunswick from August 2005 to August 2006. Gonad development occurred very rapidly in both males and females prior to spawning; there appeared to be little synchrony between fish, and gonad weight:body weight regressions were not significant for eight months of the year, including all spawning months. As many as four clutches of eggs were observed within the ovaries of female redbelly dace during May, June, July and August, suggesting that they spawn at least 7 to 10 times during the summer. While variability in some cyprinid species can be reduced by restricting sampling to specific times or certain sizes or ages, it appears that there is no simple way to reduce the variability among Northern Redbelly Dace to improve their utility as a monitoring species.

State of the Saint John River

Session Co-chairs/Présidents Kelly Munkittrick and Laura Noel

Evaluation of threats from riverine enrichment: status and effects of nutrient loading on the Saint John River, New Brunswick. ***J. Culp[1], E. Luiker[1], L. Noel[2], A. Curry[2], D. Hryn[2] and R. B. Brua[2] (PL)***

[1]Environment Canada and Canadian Rivers Institute, Fredericton, NB; [2]University of New Brunswick, Fredericton, NB

The Saint John is the largest river in the Maritimes (55,000 km^2 drainage area, ~ 700 km long) with a history of natural resource use and effluent release to the catchment since the late 17th century. These varied effluent sources modify riverine nutrient status and play a critical role in determining river productivity and metabolism. The primary aim of this study was to obtain a basic understanding of the current nutrient conditions of the Saint John River in relation to historical conditions, and to consider the effects of point and non-point nutrient loadings on ecosystem health. Our primary objectives were to: 1) determine the historical and current nutrient status of the river by acquiring historical and contemporary water quality data; 2) evaluate nutrient condition and ecological effect of nutrients in enriched areas; and 3) establish nutrient criteria for the river by developing site-specific values based on the USEPA reference condition approach and other existing methodology, followed by comparison to nutrient criteria developed for adjacent watersheds in the USA. A weight of evidence approach was used to harmonize these nutrient criteria results and to determine trophic status of river reaches, thereby highlighting areas of concern.

Measuring the effects of nutrient loading and hydroelectric discharge regulation on river metabolism using stable oxygen isotopes. L. J. Noel[1], J. M. Culp[2] and L. I. Wassenaar[3] (PL)

[1]University of New Brunswick, Fredericton, NB; [2]Environment Canada and the Canadian Rivers Institute, Fredericton, NB; [3]Environment Canada, Saskatoon, SK

River community metabolism and gas exchange rates control dissolved oxygen in rivers. Dissolved oxygen is an important parameter as it sustains aquatic life and maintains biodiversity. These processes are affected by nutrient loading and discharge regulation. Riverine communities within the Saint John River, NB, are under increasing pressure from nutrient loading and discharge regulation and it is unknown how these pressures will affect community metabolism. Along nutrient and algal biomass gradients in regulated (Florenceville) and unregulated (Edmundston) reaches of the Saint John River, community metabolism was measured during summer and fall 2004, 2005, and 2006. Metabolism of the river community was measured by a) an open-system, single station, diel measurement of dissolved oxygen and stable oxygen isotopes, and b) a dynamic O2 and δ18O-O2 model (PoRGy) that quantifies average photosynthesis, respiration, and gas exchange rates and allows predictive modeling. Dissolved oxygen concentrations were measured below CCME guidelines repeatedly. Diel patterns of dissolved oxygen exist in the Saint John River, and nutrient loading and daily desiccation caused by discharge regulation cause shifts to a more respiration-dominated community. The direct measurement of fundamental community functions such as metabolism will allow us to assess the health of river ecosystems, providing tools for ecologically sustainable management.

Investigations of oil refinery effluent on fish in the field and laboratory. J. Adams[1], D. L. MacLatchy[2] and K. Munkittrick[1] (PL)

[1]University of New Brunswick Saint John, Saint John, NB; [2]Wilfrid Laurier University, Waterloo, ON

The largest oil refinery in Canada discharges into Little River, a small estuarine stream in Saint John, New Brunswick. The refinery produces 270 000 barrels of oil/day and discharges effluent at a rate of 24 274 m3/day. Since 2003 we have been investigating the impact of refinery effluent on fish in the receiving environment. Low dissolved oxygen attributed to ballast water released with the effluent confounded efforts to assess the impacts of the effluent on fish. The recent study was initiated to assess any potential recovery after ballast water was removed from the effluent and to determine the effects of refinery effluent on fish. Potential recovery in the receiving environment has been assessed through monthly fish surveys and by comparing recent data to previous studies. Both laboratory and field studies with mummichog *(Fundulus heteroclitus)*, a brackish water species, and Northern Redbelly Dace *(Chrosomus eos)*, a freshwater species, have been conducted to elucidate any effects the refinery effluent may have on fish. Both species are resident species. The field studies with caged mummichog have shown an increase in liver size relative to fish size and a decrease in condition of the fish

downstream of the effluent discharge; female testosterone levels and liver detoxification enzymes (ethoxyresorufin -o- deethylase) were elevated. Laboratory studies with effluent and effluent with sediment with both species have not detected any differences to date. These results potentially indicate more complex reactions occurring in the field than could be simulated in the laboratory.

Issues surrounding the design of cumulative effects assessments for large rivers. K. Munkittrick[1] (PL)

[1]University of New Brunswick Saint John, Saint John, NB

Assessment of the accumulating effects of development on a watershed is a difficult and challenging task; we have been using fish and invertebrates to focus an assessment of the upper Saint John River since 1999. The methodology for this kind of effects-based assessment has been developing since the late 1980s, and it has become the dominant approach to assessing cumulative effects in academic programs. The regulatory approach to cumulative effects assessment has developed through a stressor-based exercise focused on attempting to quantify the potential impacts of a specific development, the relationship to immediately adjacent developments and potential long-term changes. We have been working on a framework aimed at conceptually incorporating elements of each approach into a holistic cumulative effects assessment framework. This approach requires the development of a level of commitment to baseline monitoring, science-based decision-making, and post-operational monitoring that is currently lacking in most situations. Some of the data needs include baseline information on ecological integrators of the accumulated environmental state, their variability, their sensitivity, and how they are changing over time. This presentation will describe some of the science-based challenges to CEA, some of the data needs and barriers to incorporating changes, and a new proposal currently under review to hold a series of workshops focused on further developing the framework.

An investigation of gonadal variability associated with wild mummichog (<u>Fundulus heteroclitus</u>) spawning activity in the lower Saint John River. V. McMullin[1], K. Munkittrick[1] and D. Methven[1] (PO)

[1]University of New Brunswick Saint John, Saint John, NB

Mummichog *(Fundulus heteroclitus)* is increasingly being used as a model species for laboratory bioassays and for monitoring the impacts of industrial effluents. They are fractional spawners (i.e., spawning adults spawn multiple times during the spawning season) which are believed to spawn between and during the months of May to September in temperate climates. Research available from the mid- and southern Atlantic coasts shows that mummichog follow a lunar cycle, with peak spawning activity corresponding to the full-moon. Field collections in 2005 suggested that the spawning period may be shorter in eastern Canada, and this study investigated the reproductive patterns of the mummichog in maritime Atlantic estuaries to determine reference

endpoints of adults and proper sampling periods during the spawning season. Minnow traps were deployed from late April until August to examine the variability associated with the spawning cycle of male and female mummichog in the lower Saint John River. The peak gonadosomatic index (GSI) for females was observed from June 9th (11.8 ±4.7) to June 29th (10.3±2.6), around the June 15th new moon. Peak male GSI also occurred during this time, with the maximum GSI peak occurring on June 12th (2.60 ±1.16). Temperature appeared to play a dominant influence on reproductive performance, with spawning starting at a temperature of 13°C. There was no evidence of a peak in reproductive activity at full moon; our results indicate some influence of the new moon on variability. Within- and between-site variability showed that effect sizes for environmental monitoring with the species need to be at least 50%; this is double that recommended for current environmental effects monitoring programs.

Environmental/Climate Change in Aquatic Toxicology

Session Chair/Président Catherine Couillard

Scaling-up spatial surveys of biomarkers for measuring the impacts of global processes in blue mussels: interaction of pollution with global warming. F. Gagné[1], T. Burgeot[2], J. Hellou[3], S. St-Jean[4], É. Farcy[2] and C. Blaise[1] (PL)

[1]Environment Canada, Montreal, QC; [2]IFREMER, Nantes, France; [3]Fisheries and Oceans Canada, Dartmouth, NS; [4]Jacques Whitford Consultants, Moncton, NB

Economic and social development has taken place at the expense of the environment's health both locally and on a global scale. In an attempt to better understand the large-scale effects of pollution and other stressors like climate change on the health status of *Mytilus edulis*, mussels were collected during the first two weeks of June 2005 at three sites (one pristine and two affected by pollution) located in each of the regions of the Canadian West Coast, the St. Lawrence estuary, the Atlantic East Coast and the northwestern coast of France, covering a total distance of some 11 000 km. The mussels were analyzed for morphologic integrity (condition factor), gametogenic activity (gonado-somatic and gonad maturation index, vitellogenin-like proteins), energy status (temperature-dependent mitochondrial electron transport activity and gonad lipid stores), defense mechanisms (glutathione S-transferase, metallothioneins, cytochrome P4503A activity and xanthine oxidoreductase), and toxic damage (lipid peroxidation and DNA strand breaks). The results showed that data from the reference sites in each region were generally not normally distributed, with discriminant factors reaching the number of regions (i.e. four), except for the biomarkers gonadal lipids, xanthine oxidoreductase lipid peroxidation in digestive gland. The integrated response of the biomarker responses revealed that biomarkers of stress were significantly more pronounced in mussels from the Seine estuary, suggesting that the impacts of pollution are more generalized in this area. Mussels from the Seine estuary and the Atlantic East Coast (Halifax Harbour) responded more strongly for vitellogenin-like proteins but were not related to gonad

maturation and gonado-somatic indexes, suggesting the presence of environmental estrogens. Moreover, these mussels displayed reduced DNA repair activity and increased lipid peroxidation. Factorial analyses revealed that energy status, cytochrome P4503A activity and vitellogenin-like proteins were the most important biomarkers. Adaptation to warmer temperatures was reflected at the energy status levels, mussels from both the polluted and warmer sites displaying increased ratios of mitochondrial activity to lipid stores. Regional observations of biomarkers of energy status, gametogenesis and pollutant-related effects were influenced by nutrition, oxygen availability (eutrophication), thermal history, and temperature.

British Columbia killer whales at risk: will climate change exacerbate contaminant risks? A. Buckman[1], P. Ross[1], C. Helbing[2], N. Veldhoen[2] and J. Ford[1] (PL)

[1]Fisheries and Oceans Canada, Sidney, BC; [2]University of Victoria, Victoria, BC

British Columbia's salmon-eating resident killer whales *(Orcinus orca)* are considered at risk from the effects of contaminants, noise and disturbance, and diminished prey. Studies have revealed that they are among the most PCB-contaminated marine mammals in the world, and are vulnerable to disruption of immune and endocrine systems. Correlations between the abundance of their preferred prey (Chinook salmon) and killer whale mortality since the 1970s highlight the critical importance of diet to resident killer whales. However, the mechanisms underlying this mortality remain unclear, and a combination of factors may weaken the health of whales and lead to increased mortality. Reductions in salmon quantity or quality associated with warmer sea surface temperatures may directly or indirectly lead to starvation and/or toxicant-associated effects in salmon-centric killer whales. In an effort to characterize the relative threats posed by climate change versus contaminant-associated toxicity, we are developing genomic tools to apply to skin/blubber biopsies that are being collected from free-ranging killer whales in British Columbia. These new techniques will assess genes that are specific to nutritional status and metabolis,m as well as contaminant-associated endocrine disruption, to assess the interplay between nutrition, contaminants, and climate change. This understanding will build on our demonstration that contaminants are affecting metabolism in harbour seals from British Columbia.

Warming the Tundra: Impact on trace gas exchange. M. Pilote[1], L. Poissant[1] and P. Constant[2] (PL)

[1]Environment Canada, Montreal, QC; [2]Institut national de recherche scientifique, Montreal, QC

The mean global surface temperature is expected to increase by 1 – 3.5 °C within the course of this century, and global circulation models indicate that warming will be the greatest at high latitudes. Several environmental factors such as temperature, precipitation, and soil moisture are known to control gas exchange. However, it is difficult to predict their response to the climate change. The main concern about the

carbon cycle is the fate of the northern peat carbon stock. The warming of the northern ecosystem would induce a release of that carbon in the form of CH4 and CO2. In summer 2006, CO2 fluxes were measured at Kuujjuarapik, on the east cost of Hudson Bay, by using a Bowen Ratio System. Net CO2 exchange over mosses was -86 mg/m²/h with a net deposition during day time, -640 ± 486 mg/m²/h, and net evasion during night time, 593 ± 639 mg/m²/h. Static Flux Chamber was also used to measure soil's respiration and CH4 exchange on different substrate during day time (~10:00 EDT). Maximum CH4 fluxes were found in Thermokarst ponds, 12.96 mg/m²/h, while the highest CO2 fluxes where measured over mosses and lichens, 56.56 and 42.31 mg/m²/h. As a whole, the Kuujjuarapik site acted as a net sink of CO2 and a net source of CH4.

Effects of oxygen depletion on blood cells of cod fish (Gadus morhua): an in vitro study. ***G. Mabrouk[1], D. Hamoutene[1] and A. Mansour[1] (PO)***
[1]Fisheries and Oceans Canada, St. John's, NL

Changes in ocean climate and seasonal development of dissolved-oxygen deficits (hypoxia) represent a challenge to ecological dynamics and fishery sustainability in coastal ecosystems around the globe. Massive kills due to hypoxic or anoxic conditions have been frequently reported for various marine species. Atlantic Cod *(Gadus morhua)* has a wide distribution in several habitats where hypoxia has been reported, especially in coastal regions such as the Gulf of St Lawrence and the Baltic Sea. To examine the in vitro effects of hypoxia on cod blood, we exposed whole blood to normoxic (20% O2, 79.8% N2, 0.2% CO2) and hypoxic conditions (2% O2, 97.8% N2, 0.2% CO2) for 3 and 24 hours. We measured lactate dehydrogenase (LDH) and catalase activities (CAT), as well as protein amounts in red blood cells and found no effect of hypoxia. Increased protein synthesis and decreased LDH were found in both normoxic and hypoxic conditions compared to baseline values (prior to the gassing process). Cell ratios and respiratory burst (RB) responses of whole blood and isolated white blood cells (WBCs) were also investigated by using cell observation and flow cytometry. No effect on RB was found after three hours of cell exposure to hypoxic conditions. Measurement of activities of other enzymes involved in oxidative metabolism is being completed.

Maritimes Leaders in Marine Ecotoxicology and Prevention

Session Co-chairs/ Présidents Bill Ernst and Jocelyne Hellou

Curiosity, physiology, and ecotoxicology. ***R. Croll[1] (PL)***
[1]Dalhousie University, Halifax, NS

The primary motivation for my own scientific career has come from a basic curiosity about animal life. Unfortunately, so-called curiosity-based research seems to receive little support from the public and politicians. Accountability and commercialization seem to be much more the buzz words of the day. Thus, society as a whole thinks that it is perfectly legitimate to sic scientists upon the latest hot question, and feels free to redefine goals

upon political whims. Often such questions are indeed pressing, but society also needs much more than answers to important questions of today. Society must also learn to value the curiosity needed to pose the important questions for tomorrow. It is only by this shift in approach that we can begin to imagine the challenges for the future. A current lack of respect or reward for curiosity also artificially fragments science into camps of applied vs. pure investigators. Scientists with applied mandates, in particular, are seldom given the room to explore important implications of their work if they fall outside the jurisdiction of prescribed governmental or industrial funding guidelines. As an academic scientist whose work ranges over fields as diverse as reproductive physiology, neurophysiology, larval biology and evolution and involves both molluscs and fish, I see numerous chances for two-way exchanges between lessons learned in aquatic ecotoxicology and my own pursuits of so-called pure science. However, those avenues for exchange are relatively few, and scientists must fight more vigorously against further fractionation of science and incursions upon curiosity-based research.

Rich Science, poor Science: Can scientists contribute to the dream of a better world?
D. Hamoutene*[1] *(PL)
[1]*Fisheries and Oceans Canada, St. John's, NL*

Even after nine years in Canada, my perspective on science is still very influenced by my upbringing in a developing country. It always seemed to me that the global community of scientists had a special responsibility to solve real-world problems and find solutions to improve the quality of life, especially in the developing world. This is particularly true for scientists concerned with pollution, since the transport and effect of pollutants is not hampered by administrative boundaries, and often the effects are strongest in areas far from the pollution source. The scientific community, sharing a long-standing tradition that transcends nations, religions and ethnicity, should promote the "intellectual and moral solidarity of mankind" as postulated in the constitution of UNESCO. The reality, however, is often markedly different: the volume of scientific research carried out in the developed world far exceeds that of developing countries and is often not directed at resolving issues faced by the developing world, even if that research is carried out in African, Asian or South American countries. In many cases, data collected in developing countries is not shared with local scientists. In addition, in much of the developed world scientists are seen to lose some measure of credibility if they venture into open discussions beyond their immediate technical expertise and participate in discussions on the broader context of global development. Why are we incapable of contributing significantly to changing the balance of wealth and prosperity? Are we too obsessed by our search for funding and need for publications to see outside the box? Are we too overwhelmed by our governing bodies to go against the status quo? Are we incapable of getting organized to make a difference? Can we still dream of a better world?

There and then back again, a transplant's tale. W. Fairchild[1] (PL)

[1]Fisheries and Oceans Canada, Moncton, NB

What path leads us to our current front door? In my case, when I was young I had a mesmerizing fascination with pond life. I never expected to pursue this as a career, and went into entomology in an agriculture faculty as a safe route to practical science and employment. My plan was that before getting serious in agriculture I could afford to do a basic apprenticeship in aquatic entomology. I never made the return to field crops and pest species, but followed aquatic interests in ponds, lakes, rivers, estuaries and the ocean. I was interested in the Maritimes geographically because, according to the Biological Survey of Canada, it has been neglected faunistically from the freshwater side for aquatic invertebrates. This is partly reasoned to be because of the strong draw of the ocean in the region, and I have succumbed to its pull and now am working more and more in the marine environment. My initial project in the Maritimes related to side effects of forest spraying, and is a good example of doing your homework, making proposals, and then having the results take you in unexpected directions. The direction was firmly down an ecotoxicological path. Returning to the Maritimes after a stay out west, some history, some insight, and a nice bit of unpublished information led to my asking a great ecotoxicological question, which turned out to be the wrong question. The right question on salmon smolts and the effects of 4-nonylphenol was far more entertaining.

Contaminants, communication and coincidence: investigating mechanisms of action of environmental estrogens in fish. S. Currie[1], N. Osborne[1] and A. J. Ward[2] (PL)

[1]Mount Allison University, Sackville, NB; [2]University of Sydney, Sydney, Australia

Synthetic environmental estrogens are now widespread in the aquatic environment, and their deleterious effects on fish reproduction, development and survival are well known. We have been examining the effects of these compounds at the molecular, physiological and behavioral levels to understand their mechanisms of action. Many of these estrogens elicit effects on fish by competing with naturally produced estrogen for ligand binding on the estrogen receptor (ER). In male and juvenile fish this activation ultimately leads to endocrine disruption, as measured by the production of vitellogenin protein (Vg). Using hepatocytes from juvenile rainbow trout, we blocked chaperone activity of the ubiquitous heat shock protein, hsp90, during exposure to the potent estrogen, 17α-ethynylestradiol. We determined that hsp90 activity is necessary for ER activation and thus endocrine disruption in fish, providing insight into the molecular mechanisms underpinning the response of fish to exogenous estrogens. From a behavioral perspective, we have shown that a 5-day waterborne exposure of juvenile rainbow trout to the exogenous estrogen, 4-nonylphenol (4-NP), reduced shoal cohesion, aggression and the ability to successfully compete for food. Furthermore, a 1 h waterborne exposure of 0.5 $\mu g \cdot L^{-1}$ 4-NP prevented killifish from detecting the presence of conspecifics, and concentrations of 1 $\mu g \cdot L^{-1}$ and above caused killifish to avoid dosed conspecifics. We conclude that low, short-term exposure to 4-NP disrupts chemical communication underlying social behaviour in fishes, ultimately resulting in negative effects on fitness.

The omega-3 cycle: microalgae, fish, man, bus. C. Barrow*[1] *(PL)

[1]*Ocean Nutrition Canada, Dartmouth, NS*

Numerous clinical trials, population studies and animal experiments have demonstrated the beneficial effects of EPA and DHA from fish oil for cardiovascular disease, brain and retinal development, and inflammatory mediated diseases such as Alzheimer's disease and depression. These fatty acids are derived from waste oil from fishmeal manufacturing, and so are a value-added product from a waste material. Several international groups such as ISSFAL, the AHA, Australian NHMRC and the British Nutrition Foundation have provided recommendations for the daily intake of EPA and DHA. Despite these recommendations, consumption is low and a nutritional gap exists in many countries. Fortification of foods with fish oil aims to reduce this nutritional gap. However, adding EPA and DHA to foods requires a stabilization technology that enables the sensory shelf life of the food to remain unchanged. Methods such as spray-dried emulsions, fluidized bed coating, liquid emulsions, liposome entrapment and complex coacervation have been used to stabilize oils rich in EPA and DHA for delivery into foods. After microencapsulation, fish oil can be formulated into food products such as bread, yogurt and orange juice so that no fishy flavor is observed for the shelf life of the food product. This presentation will describe the cycle of omega-3 from a waste product of fish meal processing, to a value-added food ingredient. It then describes the development of biodiesel utilizing a waste product from this waste product. And finally it follows the cycle back to a renewable source of omega-3 from the original microalgae that fortified the fish with omega-3.

Toxic effects of brown tide on suspension-feeding shellfish: from feeding mechanics to modeling of population effects. M. Bricelj*[1] *(PL)

[1]*Institute of Marine Biosciences, Halifax, NS*

Harmful algae encompass species that produce toxins of public health concern when accumulated in edible shellfish, as well as those which produce lesser-known bioactive compounds, such as *Aureococcus anophagefferens,* that impact grazer populations without leading to human health risk. Brown tides of this picoplankter have recurred in shallow estuaries along the US mid-Atlantic seaboard since the mid-1980s. They cause mass mortalities and recruitment failure of commercially important, suspension-feeding bivalves, as well as reduction of eelgrass beds via light attenuation. Brown tide cells produce a neurotransmitter-like compound (or compounds) that in vitro inhibits the activity of gill lateral cilia responsible for generation of feeding currents in bivalves. Use of morphologically identical toxic and non-toxic isolates has allowed controlled laboratory studies to determine ontogenetic behavioral and physiological effects of brown tide on hard clams, *Mercenaria mercenaria*, from planktonic larvae to adults. Toxic cells impair feeding but not ciliary-driven swimming of larvae. In vivo, video-endoscopy observations of adults show that toxic *A. anophagefferens* induces intermittent reversal and arrest of feeding currents and muscular convulsions of the gill within less than two

hours of exposure. Results of laboratory studies are incorporated in a biochemically-based model that is used to predict brown tide effects on hard clam populations in the natural environment.

Severe water discoloration caused by brown tide when it first occurred prevented continuation of an ongoing diving study of New York scallop populations. Our efforts were opportunistically shifted towards understanding this new phenomenon. Research often leads us in unexpected directions, and remaining open to their exploration is key to science innovation and discovery.

Metals and Metalloids

Session Co-chairs/ Présidents Christine Moore, Michael Parsons and Roxanne Razavi

Background concentrations of metals and trace elements commonly above Canadian Water Quality Guidelines (CWQGs) in natural waters of Hope Bay Belt, Nunavut. E. Jasinska[1] and G. Ash[1] (PL)

[1]Golder Associates Ltd., Edmonton, AB

Fifteen lakes and associated streams were surveyed for baseline water quality in the Hope Bay Belt area of Nunavut, as part of environmental studies associated with potential mine developments by Miramar Hope Bay Ltd. Since 1995, water quality data were collected, on and off, for about half the sites, and for most since 2004. With the exception of one lake, the concentrations of total aluminum frequently exceeded the 100 $\mu g \cdot L^{-1}$ CWQG (for waters with pH≥6.5) at all sites. When the field pH was <pH 6.5, the corresponding total aluminum concentrations were consistently above the 5 $\mu g \cdot L^{-1}$ CWQG (for pH<6.5). The above-guideline total aluminum concentrations were mostly between 100-200 $\mu g \cdot L^{-1}$ and occasionally around 500 $\mu g \cdot L^{-1}$. In addition, the lakes and streams commonly had either: total iron concentrations above the 300 $\mu g \cdot L^{-1}$ CWQG, or total selenium concentrations above the 1 $\mu g \cdot L^{-1}$ CWQG. Total selenium was usually between 1-2 $\mu g \cdot L^{-1}$. The total iron concentrations, when exceeding the CWQGs, were generally between 300-500 $\mu g \cdot L^{-1}$; however, in some of the streams, the total iron concentrations were >1000 $\mu g \cdot L^{-1}$ (up to 2760 $\mu g \cdot L^{-1}$). Total suspended solids were typically very low and there are no obvious reasons for these trends. The resident fish populations are diverse and generally in good condition. The toxicity of each aluminum, iron and selenium is discussed. It is suggested that the CWQGs for pH, aluminum and iron, and selenium should be reviewed as to their suitability for waters that naturally exceed the CWQGs for these parameters.

Speciation of arsenic and mercury in waters and sediments impacted by gold mine tailings in Nova Scotia. M. Parsons[1], G. E. Hall, C. M. Daniels[2], H. E. Jamieson[2] and S. Winch[3] (PL)

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Arsenic and mercury are present at high concentrations in historical gold mine tailings throughout Nova Scotia, and in water, sediments, and biota downstream of former mining areas. This study is a multi-disciplinary investigation of the dispersion, speciation, and fate of metal(loid)s in the environment surrounding the Upper and Lower Seal Harbour mining districts. From 1893-1941, more than 500,000 tonnes of tailings from these mines were slurried into local watercourses, and have been transported more than 2 km to the ocean where they form an intertidal tailings flat. Chemical analyses of 200 tailings and stream sediment samples show high concentrations of both As (39 $mg \cdot kg^{-1}$ to 31 wt.%; mean 1 wt.%) and Hg (12 $\mu g \cdot kg^{-1}$ to 120 $mg \cdot kg^{-1}$; mean 3 $mg \cdot kg^{-1}$). Mercury is present in elemental form (Hg0), amalgam (AuxHgx), and in secondary phases; however, dissolved Hg concentrations in surface waters are relatively low (mean 12 $ng \cdot L^{-1}$). Methylmercury concentrations are high in tailings and pore waters, but low in overlying waters. Arsenic is hosted in arsenopyrite and a variety of secondary phases such as scorodite (FeIIIAsO4·2H2O). Dissolved As concentrations are very high (0.2 to 6200 $\mu g \cdot L^{-1}$; mean 390 $\mu g \cdot L^{-1}$), as compared to background values of <2 $\mu g \cdot L^{-1}$. Measurements of As(III) and As(V) show that As(V) constitutes more than 90% of the dissolved As in most surface waters. Biological sampling by project partners has demonstrated that both As and Hg have bioaccumulated to various degrees in terrestrial and marine biota, including eels, clams, and mussels. A bivalve shellfish closure is now in effect for Seal Harbour to prevent harvesting of As-contaminated clams.

A study of thiosalt oxidation. Y. Vongporm[1], K. Hawboldt[1], C. Coles[1] and C. Bottaro[1] (PL)

[1]Memorial University of Newfoundland, St. John's NL

Thiosalts are the salts of sulphur oxyanions produced from the interaction between sulphur-rich ores and oxygen during certain mining processes. Mining wastewater typically contains a combination of thiosalt species with varying concentrations. The chemical and biological oxidation of thiosalt in natural waters produces acid which can harm aquatic organisms.

This research focuses on thiosalt oxidation and the main thiosalts studied include thiosulphate (S2O32-), trithionate (S3O62-), tetrathionate (S4O62-), and pentathionate (S5O62-). A preliminary study of thiosalt equilibrium composition was performed using HSC Chemistry software and these results were statistically analyzed by Design-Expert software. According to the analysis, the formation of thiosulphate depends on temperature, pH and initial concentration of thiosulphate. The formation of polythionate is also influenced by temperature, pH and polythionate initial concentrations. This is not

unexpected; however, under equilibrium conditions the behaviours of thiosulphate and polythionate are relatively independent of each other. The purpose of the analysis was to identify the major species involved in thiosalt behaviour in water and use these data to design experiments to determine reaction rates. As the freeze–thaw cycle has been shown to influence speciation, a stability study was conducted to determine the stability of thiosalt species under a variety of freeze and thaw conditions. At pH 2, 4 and 7, most thiosalt concentrations changed within the range of ± 6% after fast freezing in liquid nitrogen, storage in a freezer and thawing in a water bath at 20oC. Experiments were recently initiated to determine oxidation reaction rates at acidic, neutral and basic conditions at variable temperatures.

Interactive effects of metals in mixtures on bioaccumulation in the amphipod, Hyalella azteca. ***W. Norwood[1], U. Borgmann[2] and D.G. Dixon[1] (PL)***
[1]University of Waterloo/Environment Canada, Waterloo, ON; Environment Canada, Burlington, ON

Mixtures were produced of "equi-toxic" concentrations of 10 elements at the four-week LC25 for *Hyalella azteca*. Bioaccumulation was determined in one-week exposures. The first mixtures tested included seven elements: As, Cd, Co, Cr, Ni, Pb and Tl. Copper, Mn and Zn were not included in the initial tests due to potential confounding effects, such as regulation of Cu and Zn by *H. azteca* and the high concentrations of Mn required to be "equi-toxic", which might cause adsorption of metals to Mn hydroxides if these were formed. The second set of tests included the seven element mixture in combination with: Cu, Mn and Zn individually; the binary pairs, Cu-Mn, Cu-Zn and Mn-Zn; and the tertiary group Cu, Mn and Zn. Interaction factors (IF) were computed which quantified each element's impact on the bioaccumulation of the other nine. Cobalt, Cd and Ni bioaccumulation was significantly inhibited with increasing number of metals in the mixture. Arsenic bioaccumulation was enhanced with increasing number of metals in the mixture exposure. Lead bioaccumulation was enhanced by some mixture combinations. Bioaccumulation of Cr, Cu, Mn, Tl and Zn was not significantly affected by exposure to other metals.

The speciation and distribution of selenium in a contaminated lake ecosystem. ***C. I. Wiramanaden[1], E. K. Forster[1], I. J. Pickering[1] and K. Liber[1] (PL)***
[1]University of Saskatchewan, Saskatoon, SK

The Key Lake ecosystem in Northern Saskatchewan has experienced decades of selenium exposure from uranium milling. However, the spatial distribution of selenium and its partitioning among environmental compartments are not well understood. The work presented here will focus on the relationship between selenium speciation and Se concentrations in abiotic and biotic components of small lakes near the Key Lake milling site. We describe the Se distribution throughout several lakes impacted by selenium in

milling effluent. Using total Se concentrations (using ICP-MS) and analysis of chemical speciation (using X–ray absorption near edge spectroscopy), we present site-specific relationships between Se in benthos and their environment. Sediment cores, surface water, pore water and benthos were analyzed for total Se, while select samples were also analyzed for Se speciation. Lake and pore water Se concentrations showed a gradient from 18 to 1 $\mu g \cdot L^{-1}$ with distance from the discharge point. However, there was no obvious relationship between Se concentrations in benthos and those is water or pore water. Sediment Se concentration and Se speciation will also be presented in an effort to relate Se levels in benthic macroinvertebrates to Se speciation and bioavailability in sediment.

Trends in selenium concentrations in lotic waters in the Elk River watershed, British Columbia. M. Paine[1], P. Orr[2] and C. Fraser[3] (PL)

[1]Paine, Ledge and Associates, North Vancouver, BC; [2]Minnow Environmental Inc., Mississauga, ON; [3]Elk Valley Coal Corporation, Elkford, BC

The Elk River watershed in southeastern British Columbia receives drainage from five coal mining operations. Coal mining releases selenium from host rock. Trends in selenium concentrations in lotic waters from 1995 to 2006 were analyzed for 13 core stations upstream and downstream of mines on larger tributaries and the main stem of the Elk River, and for 15 stations on smaller streams receiving drainage from one or a few point sources within mines. Selenium concentrations increased significantly over time at seven of the eight core stations receiving significant mine drainage (i.e., "exposed" stations). The median rate of change in selenium concentrations for those eight exposed core stations was 8.2% per year, representing an approximate doubling of concentrations over a decade. Selenium concentrations also increased at 14 of the 15 stations on smaller streams, and the increases were statistically significant for eight stations. The median rate of change for the 15 stations was 8.7% per year. Selenium concentrations also increased by 5.5% per year between 1995 and 2006 at the federal-provincial water quality station on the Elk River downstream of all mine discharges. Collectively, these results indicate that selenium concentrations in lotic waters in the Elk River watershed have increased significantly in the last decade. However, selenium is primarily taken up from sediment and diet in lentic waters, and selenium concentrations in fish and invertebrates in lotic waters have not increased over the last decade (Orr et al., 'Selenium Concentrations in Sediment and Biota in the Elk River Watershed, British Columbia').

Patterns in selenium concentrations of sediment and biota in the Elk River Watershed, British Columbia. P. Orr[1], J. Bisset[2], J. Berdusco[2], M. Paine[3] and C. Fraser[4] (PL)

[1]Minnow Environmental Inc., Mississauga, ON; [2]Interior Reforestation Company Ltd., Cranbrook, BC; [3]Paine, Ledge and Associates, North Vancouver, BC; [4]Elk Valley Coal Corporation, Elkford, BC;

The Elk River watershed in southeastern British Columbia receives drainage from five coal mining operations. Selenium is released from host rock by coal mining and has been found at elevated concentrations in water, sediments, and biota in downstream areas

compared to reference areas. A study conducted in the spring and summer of 2006 involved collection of sediment samples and biota throughout the watershed for analysis of selenium and other substances (metals, nutrients). Samples of westslope cutthroat trout (muscle, ovary, whole body), longnose sucker (whole body), bird eggs and frog eggs were collected in the spring (May-June), and sediment, benthic invertebrates, bull trout (muscle), mountain whitefish (muscle, ovary, whole body), and vegetation were collected in summer (August). To the extent possible, samples were collected using similar methods and at previously-sampled locations to permit temporal comparisons of data, in addition to spatial evaluation of data collected in 2006.

Se was the only metal notably elevated in water, sediment, and biota in mine-exposed areas compared to reference areas. As observed in previous, smaller-scale studies conducted within the watershed, tissue selenium concentrations were higher in lentic than lotic areas exposed to mine drainage.

The upper range of background selenium concentrations measured in each sample type was computed as the mean+*t*SD (one-tailed, p=0.05) using reference area data from this and previous studies in the Elk Valley ("background benchmarks"). The background benchmarks were easy to compute, adequately reflected the upper range of background concentrations for the various sample types, and were effective in screening exposure area data to identify samples with elevated selenium concentrations. Background concentrations in the Elk Valley for sediment (4.0 $mg \cdot kg^{-1}$ dw) and whole body fish (1.7-2.1 $mg \cdot kg^{-1}$ ww) exceeded BC interim guidelines (2 $mg \cdot kg^{-1}$ dw and 1 $mg \cdot kg^{-1}$ ww, respectively). With adequate reference sample sizes, the background benchmarks could become site-specific guidelines for the Elk Valley.

Comparisons of muscle selenium concentrations to ovary and whole body concentrations resulted in strong correlations for Westslope cutthroat trout (r values >0.95) and weak correlations for mountain whitefish (r values <0.5). Therefore, muscle plug samples, which can be collected non-lethally, can be considered predictive of the selenium concentrations in other tissues of westslope cutthroat trout, but not for mountain whitefish, at least during the pre-spawning period of each species when samples were collected for this study.

Despite substantial and statistically significant increases in aqueous selenium concentrations at most areas downstream of the mines (Paine et. al., "Trends for Selenium Concentrations in Lotic Waters in the Elk River Watershed, BC", presented separately), no increases in fish or benthic invertebrate tissue selenium concentrations were evident in lotic areas over the past decade. In addition, no temporal increases were evident in selenium concentrations measured in bird or frog eggs, or lentic area fish tissues, although these data were limited to a shorter time period (<5 years).

Apparent absence of selenium impacts in the Elk River Valley, British Columbia. P. Chapman[1], R. Berdusco[2] and R. Jones[2] (PL)

[1]Golder Associates Ltd., North Vancouver, BC; [2]Elk Valley Coal Corporation, Calgary, AB

Ongoing investigations into possible effects (changes to a Valued Ecosystem Component [VEC]) or impacts (effects adversely affecting the utility or viability of a VEC) of selenium in the Elk River Valley, BC, are summarized. A management decision framework provides a means to integrate present and future information in order to effectively manage selenium releases from the coal mines and to ensure environmental protection. Studies conducted to date have determined an absence of impacts to fish (cutthroat trout) and water birds (American dipper and spotted sandpiper) living in the predominantly lotic (flowing water) areas of the Elk River Valley. In the less common but more at-risk lentic (still water) areas of the Valley, an absence of selenium-related impacts has been determined for red-winged blackbirds, eight species of waterfowl, longnose sucker and Columbia spotted frog. Two effects studies on cutthroat trout living in a lentic environment partially confirm that cutthroat trout have a relatively high tolerance to selenium. However, the two cutthroat trout effects studies also resulted in some contradictory findings. As a result, although there are no indications of impacts from selenium to cutthroat trout living in lotic or lentic areas of the Elk River Valley, a further effects study will be conducted with this species to resolve the contradictions. Monitoring studies indicate increasing concentrations of selenium in waters downstream of the coal mines but no corresponding increase in fish muscle selenium concentrations. Biogeochemical studies are being conducted into selenium release and cycling as part of ongoing management activities.

Low concentrations of selenium added to freshwater mesocosms lower mercury in fish muscle and exceed gonad selenium toxicity threshold. R. Bodaly[1] and M. Mailman[2] (PL)

[1]Penobscot River Mercury Study, Salt Spring Island, BC; [2]University of Manitoba/Fisheries and Oceans Canada, Winnipeg, MB

Selenium (Se) additions have lowered mercury (Hg) concentrations in fish muscle in past studies; however, there is controversy regarding its effectiveness without causing Se toxicity. We investigated the lowest range of Se concentrations that lower Hg in fish without introducing Se toxicity. Our null hypotheses were: 1) Se additions do not affect Hg bioaccumulation, and 2) Se additions do not impose toxicity to fish. We added sodium selenite to mesocosms at 0, 0.1, 0.2, 0.4, 0.8, and 1.6 $\mu g \cdot L^{-1}$. We also added 0.3 $\mu g \cdot L^{-1}$ of stable isotopic mercuric chloride (spike Hg). We sampled water, sediment, particulates, periphyton, zooplankton, Chironomid larvae, and yellow perch. We measured Se in fish gonads to reveal possible toxicity. Spike Hg concentrations in fish muscle negatively correlated with Se concentrations in water ($\log(y)=17.78-0.52*\log(x)$; $p=0.004$; $r2=0.88$). Therefore we rejected our null hypothesis and accepted that low

concentrations of Se in water lower Hg concentrations in fish muscle. The proportion of spike Hg in fish muscle that was methyl mercury (MeHg) was 65%, indicating that spike Hg was methylated and bioaccumulated. Spike MeHg concentrations in zooplankton, Chironomid larvae, and periphyton were not related to filtered water Se concentrations. In water, particulates, and surface sediment, spike Hg and MeHg concentrations were also not significantly related to water Se concentrations. Gonad Se concentrations positively correlated with water Se concentrations ($\log(y)=0.995+0.673*\log(x)$; $p=0.0002$; $r2=0.97$). Therefore low level additions of Se to water directly affect Se in fish gonads and elevate the risk of Se toxicity. Gonad Se concentrations exceeded the Se toxicity threshold at 1.0 $\mu g \cdot L^{-1}$ of Se in water. Selenium additions lowered Hg in fish muscle and imposed a risk of Se toxicity to yellow perch. We do not recommend low level Se additions to lower Hg in fish because of the risk of reproductive toxicity.

The role of organic matter in the mobility and bioavailability of Hg: Determining sources and fate of Hg in sediments of the Cataraqui River at Kingston, Ontario. N. Manion[1], L. Campbell[1] and A. Rutter[1] (PL)

[1]Queen's University, aKingston, ON

The Cataraqui River, which drains 930 square km of watershed before emptying into the inner harbour of Kingston, Ontario (pop: 113,000), has a long history of anthropogenic use. More than 40 industries have existed within the inner harbour over the last century, and while many of these industries are no longer present, the properties that they operated on remain to the present day as potential sources of persistent contamination. This study examined total Hg (THg) concentrations in depth profiles of 21 sediment cores within the inner harbour. The spatial distribution of Hg in the surface sediment is not homogenous, as concentrations in surface sediment adjacent to the former industrial properties, which exceed the severe effect level (>2 μg kg-1) sediment quality guideline, are significantly higher than sediment furthest from the industrial shorelines (<170 μg kg-1). Depth profiles in these areas indicate that there is a consistent, ongoing source of Hg. While Hg has not been detected in storm sewer discharge in these areas, it has been detected in terrestrial soil as high as 4300 μg kg-1. Runoff draining the highest contaminated soils indicate that erosion from terrestrial sources may be an ongoing source of Hg to sediment, as significant amounts of Hg in runoff were detected. Since the mobility and transport of Hg within sediment and the water column are known to directly influence the production of the bioaccumulative form of Hg, methylmercury (MeHg), Hg in pore water as well as the amount of organic carbon within the sediment cores will be measured. Comparisons of THg in sediment and pore water to total organic carbon and MeHg concentrations in sediment will be evaluated to determine whether Hg is being sequestered to the sediment, or whether Hg is mobile and available to biota.

Role of gas evasion from contaminated sediments as a mechanism of mercury transfer to aquatic biota. R. Razavi[1], L. Campbell[1], P. Hodson[1] and J. Ridal[2] (PL)
[1]Queen's University, Kingston, ON;[2]St. Lawrence River Institute of Environmental Sciences, Cornwall, ON

The St. Lawrence River near Cornwall, ON, has been designated an Area of Concern (AOC) due to the past deposition of mercury (Hg) from several industries for nearly a century. Recent studies have demonstrated paradoxically high concentrations of Hg in yellow perch *(Perca flavescens)* from one contaminated zone. This is puzzling, since average Hg concentrations in sediment are known to be higher at a contaminated site slightly further downstream. Studies of stomach content analyses of yellow perch show prey items exhibiting this same perplexing pattern. Extensive bubbling from contaminated sediments in the zone of interest may explain this discrepancy. The methane gas is likely produced by the decomposition of wood chips and other debris buried beneath recent sediments by methanogens and sulfate reducing bacteria. These bacteria can methylate Hg, thereby converting it to its toxic form, methylmercury (MeHg), which biomagnifies with trophic level. This study uses in situ exposure experiments with artificial substrates (rock baskets) and caging studies to determine if bubbling activity is enhancing Hg flux to biota in this zone. Artificial substrates were used to collect amphipods (Gammaridae) in high and low bubbling sites in the contaminated zone. Aufwuchs (biofilm) was collected in tandem, using tiles attached to the rock baskets. Preliminary results for both THg and MeHg indicate that benthic invertebrates collected by artificial substrates show significant differences in Hg concentrations between areas of high bubbling in the contaminated site versus areas of low bubbling in the contaminated or a non-contaminated site.

Dendrochronological and microspatial comparison of mercury in shoreline vs. inland deciduous trees. E. Siwik[1], L. Campbell[1] and G. Mierle[2] (PL)
[1]Queen's University, Kingston, ON; [2]Ontario Ministry of Environment, Dorset, ON

Mercury (Hg) research in Ontario has centered on aquatic environments, since fish can be a significant source of methylmercury for humans. Interactions between terrestrial and aquatic environments in relation to the Hg cycle have received less attention. Our research focuses on the role of trees as a source and/or sink of Hg to the environment and the importance of inland and shoreline deciduous species, including *Acer* spp. (sugar, silver and red maple), *Quercus* spp. (red oak), *Populus* spp. (eastern cottonwood), and *Salix* spp. (willow). Examination of spatial and seasonal variation of [Hg] in leaves from a variety of deciduous species indicated that average autumn leaf Hg ranged from 21-42 $ng \cdot g^{-1}$. Leaf [Hg] increased with the growing season, although there was little evidence for significant spatial trends in leaf [Hg]. Tree cores from contaminated and reference sites in the Kingston area showed that although dendrochronological techniques are not appropriate for estimating historical Hgatm, they are useful for estimating the total Hg burden of a single tree. Shoreline tree species, *Populus* and *Salix*, had the greatest bark

and wood [Hg] with maximums reaching 18 $ng \cdot g^{-1}$ This was significantly higher than more inland trees such as *Quercus* and *Acer*, with maximum values of 7 and 1.2 $ng \cdot g^{-1}$ for bark and wood [Hg]. This study will provide forest and watershed managers with a better understanding of the importance of trees in the Hg cycle and the contribution of leaf and wood Hg to litterfall and waterbodies in southern Ontario.

Analysing a long-term environmental dataset: Fish tissue mercury burden trends in Ontario. E. DeLong[1], L. Campbell[1] and G. Mierle[2] (PL)

[1]Queen's University, Kingston, ON; [2]Ontario Ministry of Environment, Dorset, ON

The Ontario Ministry of Environment (OME) has been collecting data on fish tissue Hg burdens in lakes and rivers across Ontario since the mid-1970s. It is estimated that 165,000+ fish from 86 species and 1,600+ sites have been tested for Hg, with this equating to about 1.5 million database records across Ontario. Hg burdens in fish have reportedly been declining since the mid-1970s, but many species, particularly those in higher trophic levels, still exceed Health Canada guidelines for human consumption. While the OME use their data primarily for the production of the biennial *Guide to Eating Ontario Sport Fish* and for the identification of Hg sources, the data also allow for investigation of historical spatial / temporal patterns of Hg burdens. Analysing this long-term environmental dataset is complicated largely by its heterogeneity due to non-systematic sampling methods, and presents a unique set of challenges. We have applied a model developed by the USGS to standardize the sampling characteristics of the entire database to a unique species, length and cut. We are then able to use a GIS to examine spatial and temporal trends, and associations with surrounding biogeochemistry. We present our analysis results and discuss the relevance of the trends observed to date.

Comparison of mercury concentration in different lobster and scallop tissues from the Bay of Chaleur area. Y. Guitard[1] and C. Surette[1] (PL)

[1]Université de Moncton, Moncton, NB

Mercury (Hg), a neurotoxic metal, is known to bioaccumulate and bioamplify in aquatic organisms to levels that can affect human health. Although many studies have looked at Hg bioaccumulation, effects of different metal interactions on Hg bioaccumulation mechanisms are still not well understood. This study aims at understanding metal interaction on bioaccumulation of Hg in the Bay of Chaleur region, New Brunswick. A large number of industries that discharge important quantities of metals into the environment, such as a chloralkali, a lead smelter, a coal-fired thermal plant and pulp and paper mills, are situated on the coast of the Bay of Chaleur. We sampled lobsters and scallops, two species of great importance for the fishing industry. Hg levels were determined in different lobsters and scallops tissues in order to understand its pathways and to relate them to site metal contamination. For lobsters, higher Hg concentrations were found in the tail and lower Hg concentrations were found in the female gonads.

Significant differences in Hg concentrations were found between male and female lobsters. In scallops, higher Hg concentrations were found in the digestive glands and, similarly to the lobsters, lower mercury concentrations were found in the gonads. Significant differences in Hg concentrations were observed between sites, where higher Hg concentrations in lobsters were noted in Dalhousie, an industrial site.

Health risk assessment related to consumption of fish from a proposed hydroelectric complex. R. Schetagne[1] (PL)

[1]Hydro-Québec, Montreal, QC

The proposed Romain Hydroelectric Complex in the North Shore area of Québec, Canada, calls for the impoundment of four reservoirs with a total flooded area of 220 km^2. The impoundment of these reservoirs would cause increases in fish mercury levels—by factors ranging from 3 to 8—that would last between 20 to 30 years. As part of the Environmental Impact Statement of the project, a method was developed to assess the additional health risks to three local populations consuming fish and other wildlife from the region. The approach involves the following steps: the measurement of current mercury levels in fish and other consumed wildlife; the determination of current exposure levels of the local populations, by hair mercury analysis; the determination of the relative proportion of the main components of the diet of the local populations that contribute significantly to mercury exposure, by questionnaire; the prediction of future mercury levels in the fish of the proposed reservoirs, by a mathematical model; the estimation of future mercury exposures based on the predicted fish mercury levels, the relative proportion of the components of the diet of local populations that will be affected by the project and a number of fish consumption scenarios. The preliminary results indicate very few additional health risks because the project would only affect from 0 to about 3% of the main sources of mercury exposure for the local populations.

Bioaccumulation du Cd chez le pétoncle géant et le pétoncle d'Islande: résultats de terrain et de laboratoire. C. Rouleau[1], P. Cornellier[2], C. Guillemart[2], S. St-Pierre[1] and Y. Clermont[1] (PL)

[1]Fisheries and Oceans Canada, Mont Joli, QC; [2]Institut des Sciences de la Mer de Rimouski, Rimouski, QC

En 2001, une entreprise exportant des pétoncles entiers ('princess') s'est vu refusé l'accès au marché européen parce que la concentration de cadmium (Cd) dans le pétoncle entier excédait la norme européenne de 2 µg Cd·g-1 humide (révisée depuis à 1 µg Cd·g-1 humide). Suite à cette information, quatre missions d'échantillonnage sur la basse Côte Nord ont été effectués de 2002 à 2004 afin de mesurer les concentrations de Cd dans les tissus des pétoncles géants *(Placopecten magellanicus)* et des pétoncles d'Islande *(Chlamys islandica)*. A tous les sites, la concentration moyenne de Cd était supérieure à 1 µg Cd·g-1 humide. Les concentrations de métal chez les pétoncles géants étaient plus

élevées que chez pétoncle d'Islande. La distribution tissulaire variait également selon l'espèce, 83 ± 8 % du cadmium se concentrant dans l'hépatopancréas du pétoncle géant contre seulement 35 ± 11 % chez le pétoncle d'Islande. Des expériences en laboratoire sont présentement en cours afin de mesurer la cinétique d'accumulation et la distribution du 109Cd, dissous et via le phytoplancton. Les données recueillies permettront de déterminer la route d'accumulation prépondérante chez ces animaux et elles seront comparées aux données de terrain. L'impact de niveaux élevés de cadmium sur les pêcheries commerciales de pétoncles et les travaux de recherche futurs seront discutés.

***Are coal-fired power plants affecting mercury concentrations in organisms in nearby streams?* T. Jardine[1], K. A. Kidd[2] and R. A. Cunjak[2] (PO)**

[1]University of New Brunswick, Fredericton, NB; [2]University of New Brunswick Saint John, Saint John, NB

Coal-fired power plants are known to be significant sources of mercury (Hg) to the surrounding region. However, the possibility of enhanced bioaccumulation of Hg through stream food webs due to this localized deposition has rarely been examined, despite the potential risks to nearby aquatic populations. In 2006 we examined deposition of Hg onto the terrestrial landscape and its bioaccumulation through stream food webs by collecting the lichen Old Man's Beard (*Usnea* sp.), benthic invertebrates, and fishes in a bulls-eye design at increasing distances away from a coal-fired power plant in New Brunswick, Canada. Samples were analyzed for total Hg and stables isotopes of nitrogen ($\delta^{15}N$) to determine trophic positioning. The terrestrial sentinel Old Man's Beard indicated enhanced mercury deposition within 10 km of the plant and significantly elevated concentrations as far away as 100 km. Biomagnification of Hg within streams, as measured by slopes of the Hg-$\delta^{15}N$ relationships for invertebrates and fishes, was higher at sites near the power plant, and differences in the biomagnification of this pollutant also depended on the fish species present. Blacknose dace had higher mercury concentrations than brook trout leading to greater Hg-$\delta^{15}N$ slopes in streams containing only the former species. Biomagnification of Hg was also linked to water chemistry; acidic streams with high organic matter content showed greater slopes of Hg versus $\delta^{15}N$. Future analyses will focus on separating the relative importance of water chemistry and the point source in the accumulation of Hg through these food webs to determine if the power plant may be negatively impacting aquatic populations and fish-eating consumers including humans.

Mercury and human health: A review of methylmercury in fish in sub-Saharan Africa.
E. D'Souza[1] and L. Campbell[1] (PO)
[1]Queen's University, Kingston, ON
Recent public admonition of canned tuna exceeding Health Canada's established guideline of 0.5 $mg \cdot kg^{-1}$ of mercury (Hg) in commercial fish has raised the issue of mercury and public health once again. Fish and seafood consumption is recognized globally as the primary exposure route to methylmercury (MeHg), the most toxic form of mercury to humans. According to the United Nations Environment Programme Global Mercury Assessment, in 2002, the normal Hg concentration in edible tissues of various fish species ranged from 0.05 to 1.4 $mg \cdot kg^{-1}$ wet weight. With the Hg concentrations in freshwater fish well documented in many developed countries, public health fish consumption guidelines are supported by sufficiently detailed information. However, in resource-scarce regions of the world, such as in sub-Saharan Africa, where an appreciable amount of fish is consumed as an economical source of protein, such a widespread survey has yet to be completed, even within individual countries. Recognized Hg point sources exist in sub-Saharan Africa, such as small-scale gold mining and coal combustion, as well as diffuse sources, such as biomass burning. Multiple studies of local freshwater fish have been conducted throughout the subcontinent, revealing a wide range of Hg concentration in fish. These studies will serve as the source of information for this comparative review of Hg in fish in sub-Saharan Africa versus Canada, with the goal of identifying regions of greatest concern.

Mercury concentrations in lobsters and mussels from the Belledune area, New Brunswick. M. Fraser[1] and C. Surette[1] (PO)
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The Belledune area located along the Bay of Chaleur coastline harbours numerous industries, such as a thermal plant and a zinc smelter, that are known to release heavy metals into the environment. Historically, high levels of lead, mercury and cadmium have been reported in surrounding environments of the Belledune area. The presence of these contaminants has raised concerns among the local population regarding the potential consequences on their health. To address these concerns, two population health studies were conducted in the Belledune region by the government of New Brunswick between 2003 and 2005. We did an assessment of these reports using an ecosystem approach to the environment and to human health. We found that a very small number of samples were analyzed and that types of samples are limited and give an incomplete picture of the environment. Also, some of the modeling results are questionable. We address some of these concerns by evaluating mercury levels in two types of seafood species, lobster and mussel, which are regularly consumed by local population. Twenty lobsters and 50 mussels from the Belledune region were sampled. Average mercury concentrations of 0.09 ± 0.02 $mg \cdot kg^{-1}$ ww in lobster claws and 0.038 ± 0.004 $mg \cdot kg^{-1}$ ww in mussels were observed. These Hg concentrations are similar to the ones found in the

other studies. Further analyses are needed to determine other metal concentrations in seafood. We will also establish exposure of the human population through fish consumption.

A decade later: Changes in yellow perch mercury concentrations in Kejimkujik National Park, Nova Scotia. B. Wyn[1], K.A. Kidd[1], A. Curry[2] and N. Burgess[3] (PO)

[1]University of New Brunswick Saint John, Saint John, NB; University of New Brunswick, Fredericton, NB; [3]Environment Canada, St. John's, NL

Studies in 1995 and 1996 showed that loons in Kejimkujik National Park, Nova Scotia had the highest mercury (Hg) concentrations of any population in North America which were due to the high Hg concentrations in their main prey, yellow perch *(Perca flavescens)*. In 2006 we revisited 10 of these acidic lakes to quantify the current concentrations of Hg in yellow perch and determine whether there have been any decreases in this pollutant as a result of reduced atmospheric deposition of acidifying substances. In both the 1996 and 2006 studies, nine yellow perch from each of three size classes (5-10 cm, 10-15 cm, and 15-20 cm) were captured from the lakes in late summer, measured for length and weight, and scales were removed for ageing. Water quality data were also collected every year from these systems. In each lake, t-tests were used for each size class of perch to determine whether Hg concentrations were significantly different among years. Regressions were used to assess water chemistry changes through time and to relate the perch Hg concentrations to the chemical characteristics of each lake. In 1996, mean yellow perch Hg concentrations ranged from 0.11 in the smallest size class to 0.51 $\mu g \cdot g^{-1}$ wet weight in the largest class, and current concentrations ranged from 0.15 to 0.57 $\mu g \cdot g^{-1}$ wet weight for the same groups. T-tests revealed significant increases in Hg concentrations in 3 lakes for the 5-10 cm size class, eight lakes for the 10-15 cm size class, and no lakes for the 15-20 cm perch. The largest change occurred in the 5-10 cm perch at Beaverskin Lake, where the mean concentration tripled from 0.11 to 0.31 $\mu g \cdot g^{-1}$ wet weight between 1996 and 2006. Thus, although aqueous sulphate concentrations in these lakes have significantly declined over the past decade, there has not been a concurrent decrease in Hg contamination of the Kejimkujik food webs.

Deformity evaluation in northern pike and white sucker exposed to uranium mining and milling effluent. J. Muscatello[1] and D. Janz[2] (PO)

[1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]University of Saskatchewan, Saskatoon, SK

Effluents from uranium mining and milling operations generally contain elevated concentrations of various metals (e.g., U, Ni, As, Se, Mo) and ions (e.g., sulfate, ammonium). Our previous work showed elevated deformities in northern pike exposed to uranium milling effluent at a site in operation for more than twenty years. The objective of the present study was to evaluate potential selenium-induced terata (craniofacial, skeletal, and fin deformities) and edema in northern pike *(Esoxlucius)* and white sucker

(Catostomus commersoni), exposed to effluents from a uranium mine in operation for less than ten years. Spawning fish were collected from a reference site and an exposure site located downstream of effluent discharge. A two-way ANOVA design was employed with embryos originating from reference and exposure sites incubated in both reference and exposure water. Mean egg selenium concentrations were significantly higher in pike (8.02 $\mu g \cdot g^{-1}$ (dry weight)) and white sucker (4.89 $\mu g \cdot g^{-1}$ (dry weight)) from the exposure site compared to reference site (2.35 $\mu g \cdot g^{-1}$ and 1.94 $\mu g \cdot g^{-1}$, respectively). Among all evaluated deformities, only edema in white sucker fry was significantly elevated compared to the reference site. Our results are consistent with published selenium tissue thresholds for early life stage abnormalities in other fish species, with selenium accumulation in pike and white sucker collected at this site below the 10 $\mu g \cdot g^{-1}$ egg threshold associated with elevated deformities.

Selenium accumulation and effects in aquatic organisms downstream of a uranium mining and milling area in northern Saskatchewan, Canada. J. Muscatello[1], D. Janz and A. Belknap[1] (PO)

[1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK

Elevated concentrations of selenium have been reported in water, sediments and fish tissues downstream of uranium mining and milling operations in northern Saskatchewan, Canada. Dietary, as opposed to aqueous, exposure is the most important route of selenium exposure in aquatic organisms. A major implication of elevated environmental selenium levels is its propensity to bioaccumulate through aquatic food webs, resulting in potential adverse effects (e.g., impaired reproduction) on top predatory fish. The objective of the present study was to determine total selenium concentrations in all major compartments of the aquatic ecosystem downstream of a uranium milling operation, and to correlate these with the appearance of selenium-induced deformities in a native fish species, northern pike *(Esox lucius)*. Water, sediment, plankton, periphyton, invertebrates and small-bodied fish samples were collected from a reference lake and two exposure lakes, all within the same watershed. Selenium concentrations were consistently higher for all trophic groups from both exposure sites in comparison to the reference site. Our results suggest that selenium was accumulated through the food chain even though the water concentrations were low (approximately 0.8-2.6 $\mu g \cdot L^{-1}$). The bioaccumulation of selenium in prey items at the exposure sites was sufficient to result in elevated selenium-induced larval deformities in northern pike, a top predatory fish species.

The effect of sodium selenite and selenomethionine on head kidney function in rainbow trout. L. Miller[1] and A. Hontela[1] (PO)

[1]University of Lethbridge, Lethbrdge, AB

Selenium (Se), an essential element, can be toxic a concentrations slightly greater than those needed to maintain homeostasis. The different forms of Se have different toxicities and selenite appears more toxic than selenomethionine in vivo. Cortisol, a steroid

synthesized by the head kidney in teleosts, is synthesized in response to an internal or external stimulus the fish perceives as a stressor. It is responsible for a series of physiological changes that allow fish to maintain homeostasis.
The objectives of this study were to determine if (1) selenium compounds impair head kidney cell function and (2) sodium selenite is more toxic than selenomethionine to rainbow trout head kidney cells. Primary cultures of juvenile rainbow trout *(Onchorhynchus mykiss)* head kidney cells were exposed to sodium selenite or selenomethionine for one hour in a dose response study. Cell viability was assessed using a lactate dehydrogenase in vitro toxicity kit and trypan blue, while cell function was assessed by measuring cortisol secretion after a one hour stimulation with ACTH. Exposure to sodium selenite inhibited cortisol secretion in a dose dependant manner, but did not alter cell viability. This suggests that Se, in the form of sodium selenite, can impair cortisol secretion. The effect of selenomethione on head kidney cell function and viability will also be discussed. (Funded by MITHE-RN and Alberta Ingenuity)

Comparing metal-gill binding with toxicity when rainbow trout (Oncorhynchus mykiss) are exposed to metal mixtures. ***A. Winter[1], R. C. Playle[1], D. G. Dixon[2], U. Borgmann[3] and M. P. Wilkie[1] (PO)***
[1]Wilfrid Laurier University, Waterloo, ON; [2]University of Waterloo, Waterloo, ON; [3]Environment Canada, Burlington, ON

Multiple metal-gill modeling based on the toxic unit concept suggests that metals with the same toxic actions (e.g. Pb and Cd interacting at Ca-gill channels) will have strictly additive toxic effects if the concentrations of the two metals sum to one toxic unit. Due to the non-linear nature of the models, the metal mixture will be more than strictly additive below one toxic unit and less than strictly additive above one toxic unit. This research tests this model against reality by exposing juvenile rainbow trout (~1 g) to mixtures of Cd and Pb. Previous research revealed that mixtures of Pb and Cd often result in higher gill-Pb than gill-Cd, even though Cd has a stronger binding affinity to trout gills than Pb. To investigate how Pb affects Cd binding to fish gills, trout were exposed to a range of Cd concentrations (0.8, 1.5, 2.2 and 3.0 μM) while maintaining constant Pb concentrations. At the lowest metal concentrations (0.8 μM Cd and Pb), gill-Pb and gill-Cd were about equal suggesting strict additivity. Also, gill-Cd exceeded gill-Pb when 0.8 μM Pb was added to higher concentrations of Cd. 96 h toxicity experiments were also performed to investigate how toxicity compares to metal-gill binding and no correlation was evident.

Fungal growth in a heavy metal precipitating spring in central Germany. G. Krauss[1], J. Ehrman[2], F. Bärlocher[2] and G. Krauss[2] (PO)

[1]Helmholtz Centre for Environmental Research, Leipzig, Germany; [2]Mount Allison University, Sackville, NB

Plant litter decomposition is a key process in aquatic ecosystems. Fungal decomposition allochthonous organic matter is a crucial function of stream biota involved in the energy flow within food webs. Fungal growth on exposed alder leaves was studied in two heavy metal polluted streams in Central Germany. This area has a long history of copper shale mining and smelting. Ergosterol analyses indicated that neither habitat was an optimal habitat for fungi, but leaves exposed at the less polluted site (H8) decomposed rapidly and were colonized externally and internally by fungi and other microorganisms. Leaves exposed at the highly polluted site (H4) decomposed very slowly and fungal colonization was restricted to external surfaces. An amorphous organic layer, deposited within 24 hours of exposure, quickly became covered with a pale blue-green crystalline deposit (zincowoodwardite) with significant amounts of Al, S, Cu and Zn, determined by energy dispersive x-ray spectroscopy (EDS). Scanning electron microscopy analysis (SEM) revealed a branching arrangement of the precipitated particles associated with fungal hyphae growing on the surface. Undisturbed hyphae were usually completely encased in the precipitate, but hyphae did not contain EDS-detectable amounts of precipitated metals. Growth of mycelia continuously generates new substrate and therefore thicker layers of precipitate on leaves compared to artificial substrates.

Effects of Pb and Cd mixtures on gill function in rainbow trout (Oncorhynchus mykiss). O. Birceanu[1], P. L. Gillis[2], J. McGeer[1], J. Chowdhury[3], C. M. Wood[3] and M. P. Wilkie[1] (PO)

[1]Wilfrid Laurier University, Waterloo, ON; [2]University of Guelph, Guelph, ON; [3]McMaster University, Hamilton, ON

Freshwater fishes found in the ion-poor, low pH waters of the Canadian Shield are more likely to be exposed to metal mixtures rather than individual metals. We therefore examined survival, gill metal accumulation, and ionoregulation in juvenile rainbow trout exposed to Pb and Cd in soft (pH 6.0) water. The respective 96 h LC50s for Pb and Cd were 1.2 μM and 5.7 nM. Because both metals are Ca analogs, we predicted that Pb-Cd mixtures would lead to additive gill-metal accumulation at low metal concentrations, and that Cd would out-compete Pb for gill-binding sites due to its greater binding affinity for Ca-channel sites. Although Pb and Cd gill-binding was additive, Cd did not competitively inhibit Pb-gill binding. In fact, Pb-gill binding was greater, suggesting there were additional Pb-gill binding sites. This was confirmed when Pb inhibited gill Na influx, presumably by acting upon cytosolic and/or basolateral membrane targets, even at high external Cd concentrations. Predictably, Ca influx inhibition was greater in the presence of both metals, confirming that Pb and Cd compete with Ca for Ca-channel access. We conclude that Pb and Cd compete with Ca for gill Ca-channel access, but not with each other. Supported by NSERC and the MITHE-RN.

Determination of trace metal in water: Sampling, clean environment and analysis by ICP-MS. ***N. Dassylva[1], A. Tremblay[1], G. Guay[1], G. Labbé[1], S. Morissette[1], D. Thomassin[1], S. Cloutier[2] and D. Berryman[2] (PO)***

[1]Centre D'expertise En Analyse Environnementale Du Québec, Quebec, QC; [2]Ministère du Developpement durable, de l'environnement et des parcs, Quebec, QC

Analysis of trace metals in water is conducted by CEAEQ in accordance with a rigorous protocol that includes the decontamination step of the sampling bottle, the sampling procedure and the analysis by ICP-MS in a clean room. All of these steps are very important, because the detection limits obtained are the results of precautions taken to eliminate the outside contamination. The plastic sampling bottles are decontaminated by successive soaking in different concentrations of acid solutions and then washing with ultra-pure water many times. After that, the material is inserted into polyethylene bags, before use. The sampling step requires precautions like wearing gloves and needs the presence of two people. Field and shipping reagent blanks come with the samples to insure the absence of contamination during the sampling process. The following metals are analysed: silver, aluminium, copper, iron, mercury, manganese, molybdenum, nickel, lead, antimony, selenium, strontium, uranium and zinc. The detection limits varied to 0.0008 $\mu g \cdot L^{-1}$ for uranium up to 0.7 $\mu g \cdot L^{-1}$ for zinc. In the province of Quebec, la Direction du suivi de l'état de l'environnement conducted a monthly monitoring program for ten rivers a year. The background results of 26 rivers have been documented since 2004.

Uranium uptake and accumulation via adsorbed and absorbed dietary exposure routes in the freshwater amphipod, <u>Hyallela azteca</u>. ***J. Hunt[1] and K. Liber (PO)***

[1]Toxicology Center, University of Saskatchewan, Saskatoon, SK

Delineating and quantifying the routes of metal uptake in freshwater invertebrates are important for understanding metal bioaccumulation and toxicity. However, the dietary route of metal accumulation and toxicity in freshwater invertebrates has, until recently, largely been overlooked. Some researchers have assumed aqueous exposure to be the major route of uptake, while others have suggested that the dietary route can be a significant contributor to total bioaccumulation of metals in benthic invertebrates. The uranium (U) mining and milling industry in northern Saskatchewan, Canada, can lead to localized increases of U in aquatic systems due to release of effluent. We investigated the dietary bioaccumulation of U in *H. azteca* juveniles fed either Nutrafin® fish food (with adsorbed U) or dried *Chlamydomonas reinhardtii* (with absorbed U), prepared to contain 100 and 1500 mg $U \cdot kg^{-1}$ d.w. The experiment was 10-d in duration, with complete water and substrate renewal on day five to keep the aqueous concentration of U low. Test endpoints included growth, survival, and U bioaccumulation. Since low levels of U were found in the exposure water (desorption from the food), the aqueous contribution to total U bioaccumulation was investigated separately using a water-only study with a similar test design, and aqueous U concentrations based on those measured in the dietary experiment (2 and 20 $\mu g \cdot L^{-1}$ for Nutrafin® exposure, and 6 and 60$\mu g \cdot L^{-1}$ for *C. reinhardtii* exposure). Dietary U bioaccumulation resulted in growth inhibition and

decreased survival in animals fed 1500 mg $U \cdot kg^{-1}$ of either food type, but not 100 mg $U \cdot kg^{-1}$. Excess food was noted at 1500 mg $U \cdot kg^{-1}$ of either food type, but not 100 mg $U \cdot kg^{-1}$. No growth or survival effects were observed from the aqueous exposures. The dietary U contribution from each experiment was calculated and compared to show a difference in bioaccumulation between adsorbed or absorbed uranium.

Elemental mercury interaction with lake sediments. A. Bouffard[1] and M. Amyot[1] (PO)
[1]University of Montreal, Montreal, QC

Elemental mercury (Hg(0)) may be a substrate for methylation by sediment bacteria, potentially leading to the formation of neurotoxic methylmercury. However, there currently is no published data on aqueous Hg(0) concentrations in sediment porewaters. The purpose of this research was to examine Hg(0) interaction with lake sediments. Specifically, we tested the influence of chemical and biological variables on Hg(0) adsorption. Our main finding was that aqueous Hg(0) can easily and rapidly be adsorbed by sediments. Sediments were sampled at various depths (3.5, 6 and 8.5 m) in Lake Croche (45°56′ N, 74°00′ W) and amended with a solution of aqueous Hg(0), under laboratory conditions. Dissolved oxygen concentration in sediments, pH and bacterial activity had little or no influence on the level of Hg(0) adsorption on sediments particles. Conversely, sediment composition significantly influenced their adsorption capacity. As their sampling depth increased, sediments showed an increasing adsorption capacity, probably indicating an influence of granulometry and organic matter content. However, sediments sampled at a depth of 8.5 m displayed a drastically reduced affinity for Hg(0). These sediments were collected below the thermocline and in anoxia. This reduced affinity could thus be attributed to a different source of organic matter to the sediments and/or to a modification of chemical conditions. The large adsorption capacity of sediments towards Hg(0) could indicate that a significant portion of total Hg in sediments is adsorbed Hg(0). Preliminary analyses of Hg speciation on sediment particles support this assertion. Our results tend to indicate that Hg(0) may be a common Hg species in sediments and future studies regarding its bioavailability towards bacteria are warranted.

Examination of metal contamination within the Rideau River waterway. S. LeBlond[1], K. Hamilton[1], A. Rutter[1] and L. Campbell[1] (PO)
[1]Queen's University, Kingston, ON

The Rideau River waterway is comprised of a series of lakes connected by 175-year old locks linking the Ottawa River in Ottawa and the Cataraqui River in Kingston. A preliminary study evaluated metal concentrations in nine lakes between Smith Falls and Kingston. Cu, Co, Ni, Cd, Pb and Zn sediment metal concentrations were highest in Upper Rideau Lake (1.6 to 208.9 $mg \cdot kg^{-1}$) and Indian lake (2.7-287.7 $mg \cdot kg^{-1}$). Metal concentrations were lowest at Lilla Burke Park in Kingston (0.6-22.79 $mg \cdot kg^{-1}$). Elevated concentrations of Cu, Co, Ni, Zn and Rb (0.003-0.073 $mg \cdot L^{-1}$) were found in water, but were not correlated with sediment metal concentrations, nor with sediment grain size (in

all but one instance). Sediment organic content and metal concentrations were significantly correlated for Cu, Co, and Rb (p-values 0.0290, 0.0095 and 0.0013, respectively). There were significant correlations between Hg and Cr and between Cd, Pb, and Zn sediment concentrations across locations ($p < 0.001$). While Hg was not detected in tested waters, it ranged between 0.160 and 0.223 $mg \cdot kg^{-1}$ in sediments for Upper Rideau Lake and Indian Lake, respectively. The MOE's records indicate that average Hg concentrations in sport fish from Indian Lake (0.789 $\mu g \cdot g^{-1}$) are almost double those from Upper Rideau Lake (0.356 $\mu g \cdot g^{-1}$). Results from additional sediment coring and future management implications for the Rideau River Waterway will be discussed.

Status of mercury and metals in Eastern China and associated human risks. T. Fang[1], L. Campbell[1], L. Cole[1], W. Chan[1] and Y. Wang[1] (PO)

[1]Queen's University, Kingston, ON

Due to the rapid industrial development and the importance of freshwater fish in the Chinese diet, total mercury (THg) and other metals in fish are of increasing concern. In May 2005, two species of catfish *(Silurus meridionalis* and *Mystus nemurus)* and two species of carp (*Sristichthys nobilis* and *Ctenopharyngoden idellus)* were sampled at local fish markets near four Chinese lakes: Tai Lake and Dongting Lake, large, shallow, highly industrialized water bodies; Qiandao Lake, a deep man-made reservoir, and Xinshan Lake, a smaller lake south-west of Tai Lake. Mean THg concentrations, in $mg \cdot kg^{-1}$, were observed in the following order: Qiandao Lake (0.688) > Xinshan Lake (0.615) > Dongting Lake (0.173) > Tai Lake (0.071). The total Hg in fish from Qiandao Lake and Xinshan Lake was above the Chinese contamination limit (0.300). In May 2006, a deeper study measured the concentration of THg and other metals (Cr, Cu, Co, Ni, Cd, Pb, Zn, Rb, Cs) in dorsal samples from two species of wild fish, southern catfish *(Silurus meridionalis)* and yellow catfish *(Pelteobagrus fulvidraco)*, and two species of farmed fish, bluegill *(Lepomis macrochirus)* and channel catfish *(Ictalurus punctatus)* at Qiandao Lake. The THg concentrations of southern catfish (1.917) and yellow fish (0.629) were above the Chinese contamination limit. At this level of contamination, frequent consumption of these fish could be detrimental to human health. Thus, the impact and extent of metal contamination on human health around Qiandao Lake and other studied lakes will be our next focal point since many commercial fish are farmed there. Using current data to evaluate the human health risk for Qiandao Lake and other regions is of practical significance.

Measures of oxidative stress and histopathology in juvenile northern pike (Esox lucius) downstream of a metal mine. J. Kelly[1] and D. Janz[1] (PO)

[1]University of Saskatchewan, Saskatoon,SK

Lakes receiving effluent from the Key Lake uranium mill in northern Saskatchewan contain elevated metals, some of which are reported in the literature to be associated with increases in reactive oxygen species in cells and tissues causing oxidative stress. The potential for oxidative stress was assessed in juvenile northern pike collected from two exposure (high and low) and one reference lake near the Key Lake operation. The concentrations of total (t-GSH), reduced (GSH) and oxidized glutathione (GSSG) and the ratio of oxidized to reduced glutathione (GSSG:GSH) did not differ significantly among exposure and reference pike liver and kidney, with the exception of low exposure pike kidney that had significantly elevated GSSG and GSSG:GSH. The concentration of by-products of lipid peroxidation (malondialdehyde and 4-hydroxyalkenals) was significantly elevated in reference versus high and low exposure kidney and in reference versus high exposure liver. The activity of the antioxidant enzyme glutathione peroxidase was greater in high exposure than reference liver. Histopathological evaluations of liver, kidney and gills showed greater kidney and gill pathology in reference lake pike, whereas for liver, hepatocyte morphology differed among lakes without any clear signs of pathology. Trace metal analyses of muscle showed that eight elements (As, Co, Cu, Fe, Mo, Se, Tl, U) were significantly elevated in exposure pike. These results provide only limited evidence of oxidative stress in exposure pike tissues and no evidence of histopathology despite indications that metals are bioaccumulating in tissue.

Stable Isotopes in Ecotoxicology

Session Co-chairs/ Présidents Ève Dussault and Tim Jardine

Linking volcanoes and fish: Metal biomagnification in Patagonia Andean lakes. L. Campbell[1], M. Arribére[2], J. Revenga[2], R. Daga[2] and S. Guevara[2] (PL)

[1]Queen's University, Kingston, ON; [2]Centro Atómico Bariloche, Argentina

Volcanic activity is an important natural source of trace elements to the environment globally. While increased concentrations of metals and other trace elements in non-biotic aquatic environments have long been associated in areas impacted by recent volcanic activity, there has been very little research directly assessing the relationship between volcanic eruptions within a region and the biomagnification of volcanic elements through aquatic food webs. Nahuel Huapi National Park (NHNP), located on the Argentina side of the northern Patagonia Andes range, encloses a 700 km2 drainage basin with three major river systems and numerous lakes. In more recent years, the NHNP lakes have been directly impacted by the Cordòn Caulle-Puyehue volcano eruptions just across the

border in Chile (1920-21 and 1960). The Cordòn Caulle-Puyehue volcanoes and other Andean volcanoes in the region are known to be enriched in alkali elements (e.g., Rb, Cs, K), which have the potential as bioaccumulative biogeochemical tracers to assess the importance of metals derived from volcanic activity to aquatic biota. Rubidium (10-70 $mg \cdot g^{-1}$) and cesium ($0.2 - 2.0\ mg \cdot g^{-1}$) were found to biomagnify consistently in all NHNP lakes, with top trophic fish having among the highest Rb concentrations globally. We present data on food web biomagnification of key elements using stable isotopes of N and C to quantify trophic transfer. We then discuss how Rb and Cs in Nahuel Huapi lakes provide a unique opportunity to link biomagnification trends in food webs from natural sources (as opposed to anthropogenic sources).

Use of stable isoptopes and radioisotopes in pulp and paper EEM Investigation of Cause studies: Four examples from British Columbia. M. Davies[1], N. Mehlenbacher[1], M. Ptashynski[1], J. Wilcockson[1] and W. Gibbons[1] (PL)
[1]Hatfield Consultants Ltd., Vancouver, BC
Potential biological effects observed in the vicinity of pulp mills in Environmental Effects Monitoring (EEM) programs may result from current mill operations, historical mill operations and discharges, or other natural or anthropogenic factors unrelated to pulp mill operations. In EEM studies where previous investigations have demonstrated potential effects on biota, Investigation of Cause (IOC) studies are conducted to distinguish among potential sources of the observed effect. Examination of stable isotopes and radioisotopes in receiving environments of pulp mills offers a potentially powerful tool to assist in this discrimination. Hatfield Consultants examined radioisotopes of carbon and nitrogen in four EEM IOC studies (three marine and one freshwater) in EEM Cycle Four, to identify sources of organic matter and contaminants in sediments and biota in the vicinity of these pulp mills, track organic matter through benthic food webs, and attempt to identify sources of organic matter in affected biological communities. Stable isotopes were analyzed in conjunction with other chemical endpoints and, in one case, radioisotopes in sediment cores, to understand deposition of organic matter and contaminants in sediments since the mill began operation in the 1950s. Results of these studies are presented, with specific reference to the utility of stable isotope and radioisotope data to understanding and discriminating among multiple potential causes of effects observed in biological monitoring studies.

Prospects and challenges in using stable isotopes to measure baseline contaminant concentrations and biomagnification in streams. T. Jardine[1] and K. A. Kidd[1] (PL)
[1]University of New Brunswick Saint John, Saint John, NB
Stable isotope analysis (SIA) has become a useful tool in a variety of ecotoxicological applications, including assessments of the movement of contaminants through aquatic food webs. While numerous studies have used SIA to quantify the trophic transfer of persistent pollutants such as mercury or organochlorines through lacustrine food webs, this technique has rarely been applied to streams. In particular, SIA of nitrogen ($\delta^{15}N$) appears to provide important information about baseline contaminant concentrations and biomagnification through freshwater food webs, as measured by the intercepts and slopes of the contaminant vs. $\delta^{15}N$ regressions, respectively. However, there are several caveats

and considerations involved with this approach that complicate its use in stream systems. First, intercepts of contaminant- $\delta^{15}N$ relationships are sensitive to differences in the $\delta^{15}N$ values at the base of the food web across systems. While baseline $\delta^{15}N$ can be standardized by collecting and analyzing long-lived primary consumers such as shellfish, these organisms are rarely found in running waters. In addition, the seasonal variation in the $\delta^{15}N$ of the available primary consumers (such as mayflies that are more commonly found in streams) may be so large that a single sample may not be appropriate as a baseline. Finally, the contaminant-$\delta^{15}N$ slopes may be driven largely by the characteristics of the species at the top of the food chain; some fish species have higher contaminant concentrations for a given trophic level than others. We illustrate these potential confounding issues with examples New Brunswick streams, where $\delta^{15}N$ is being used to measure biomagnification of mercury, and offer some solutions and avenues for future study.

Use of archived fish tissue to determine long-term effects of exotic species on food web structure and Hg biomagnification of Lakes Nipigon, Simcoe and Champlain. J. Poulopoulos[1] and L. Campbell[1] (PL)

[1]Queen's University, Kingston, ON

Exotic species have altered food web structures and exposure of native species to environmental contaminants in many aquatic ecosystems. In particular, Lakes Nipigon, Simcoe, and Champlain represent a gradient of impacts from remote, relatively pristine northwestern Ontario, to the more urban, impacted southern Ontario and northeastern USA. Archived fish held in museums can reveal historical environmental conditions and long-term impacts of exotic species on lake health. We are therefore performing stable isotope analyses on fish collected as early as the 1920s—before the arrival of most exotic species to our study lakes—and on more recently captured fish, to determine how exotics have changed food web structures over the last 80 years. Results to date indicate a well-conserved stable isotope composition among lower trophic levels through time, but changes at higher levels that may be due to the energetic pressures of exotic species. As part of this project, the concentrations of the biomagnifying chemical methylmercury are being measured at different trophic levels. Through analysis of archived fish, it may be possible to evaluate historical methylmercury concentrations, assuming they have not been altered by chemical preservation. We are investigating this possibility and expect to be able to compare methylmercury biomagnification in historical and contemporary fish, and gauge whether, by altering trophic structures, introduced species have changed patterns of mercury contamination.

The role of invasive Bythotrephes in inland lake food webs and mercury trophic transfer to fish. E. Hatton[1], S. Arnott[1] and L. Campbell[1] (PL)

[1]Queen's University, Kingston, ON

Bythotrephes longimanus, a predatory cladoceran, has spread rapidly to numerous lakes through the Great Lakes region. Post-invaded lakes had reduced zooplankton species richness, biomass and altered community structure. *Bythotrephes* may also affect the diet and trophic position of an important macroinvertebrate prey species for fish, *Mysis relicta*. However, there has been little research on the bottom-up food web effects of *Bythotrephes* invasion, particularly in relation to contaminant biomagnification. In 2006 we collected zooplankton, invertebrate predators, forage and piscivorous fish from invaded Peninsula and Harp lakes in south-central Ontario. Mercury (Hg) and stable isotope values (δ13C, δ15N) were measured. Based on δ15N values, *Bythotrephes* shared a similar trophic position to native macroinvertebrate predators (9 and 7‰ for Peninsula and Harp, respectively). Using a mixing model (IsoSource) we show that, despite low and patchy abundance, *Bythotrephes* may be a key prey item to fish, with approximate dietary contribution of 14% (range: 0-70%) for forage fish and 10% (0-64%) for piscivorous fish. After mixed zooplankton (mean, 45%), mixing model results indicated *Chaoborus* (mean, 12%), and *Mysis* (mean, 6-9%) are also isotopically important dietary items to forage fish. In both lakes, Hg conformed to predicted biomagnification trends as indicated by δ15N. In lakes already containing native macroinvertebrate predators, the addition of *Bythotrephes* may influence the food web in terms of energy flow and Hg bioaccumulation.

Mercury accumulation in acidic versus circumneutral systems. B. Wyn[1], K.A. Kidd[1], A. Curry[1] and N. Burgess[3] (PL)

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Mercury (Hg) concentrations in fish from acidified lakes (pH < 6.0) are typically elevated above those from circumneutral systems. Hg concentrations increase up food webs and are highly correlated with trophic position (as determined by stable nitrogen isotopes; δ15N), so the slope of the Hg-δ15N regression can be used to quantify the bioaccumulation of this pollutant in lakes. In this study, Hg-δ15N slopes and food web structures in four acidified lakes (pH 4.5 – 5.5) in Kejimkujik National Park, Nova Scotia, were examined to determine whether atypical Hg bioaccumulation occurs in low pH systems and explains the elevated Hg concentrations in the top predators, yellow perch. Littoral, pelagic, and profundal invertebrates and yellow perch, golden shiner, banded killifish, and brown bullhead were captured in 2006 from each lake and analysed for MeHg (invertebrates) or total Hg (fish). δ13C and δ15N were also measured in all organisms to determine food source and trophic position, respectively, and to assess whether the structures of these acidified food webs differed from those in circumneutral systems. Mean Hg concentrations in yellow perch ranged from 0.22 - 0.52 $\mu g \cdot g^{-1}$ wet

weight among lakes and were positively correlated with trophic position within lakes. Although perch Hg varied significantly among lakes (standardized for length), δ15N and δ13C analyses showed that their mean trophic level (3.57 to 4.06) and reliance on littoral carbon (71.8 - 100%) were similar across all systems. Hg - δ15N regressions will be used to quantify bioaccumulation of this pollutant through these food webs and to contrast to data from circumneutral systems.

Environmental Risk Assessment

Session Co-chairs/ Présidents Charlene Burnett, Lisa Marshall, Malcolm Stevenson and Rob Willis

The new national protocol for the Derivation of Canadian Water Quality Guidelines for the Protection of Aquatic Life. ***U. Schneider[1], K. Potter[1], M. Demers[1], J. Hill[1], T. Fletcher[2], I. Guay[3], R. Casey[4], N. Nagpal[5] and S. Roe[1] (PL)***

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The Canadian Council of Ministers of the Environment's Water Quality Task Group comprised of provinces, territories, and the federal government has revised the protocol for the derivation of Canadian Water Quality Guidelines for the protection of aquatic life (CWQG-PAL) (CCME1991) with input from other experts.

CWQG-PAL will preferentially be derived using a distribution-based statistical analysis of all relevant toxicological data (species-sensitivity distribution). Where possible, the influence of toxicity modifying factors (e.g., pH, hardness) will be incorporated into the recommended water quality guideline values, thereby allowing for more situational variability and site-specificity. The CCME 1991 (i.e., the lowest scientifically-defensible effects concentration divided by a fixed safety factor.methodology) will still be used where data are limited. CWQG for short-term exposure will also be derived in addition to the traditional long-term exposure values. This presentation will outline the procedures for deriving a water quality guideline under the new protocol and the rationale behind some of the decisions taken.

Environment Canada and Pest Management Regulatory Agency: Environmental management of pesticides through complementary processes. E. Sabo[1], P. Jiapizian[1], M. Demers[1] and S. Kirby[2] (PL)

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The Pest Management Regulatory Agency (PMRA) of Health Canada is the federal agency responsible for the regulation of pest control products in Canada. As the federal authority under the Pest Control Products Act (PCPA), the PMRA develops pest management policies and guidelines. The National Guidelines and Standards Office of Environment Canada also develops pesticide management guidance, in the form of Canadian Water Quality Guidelines (CWQGs). These CWQGs are published through the Canadian Council of Ministers of the Environment and are voluntary measures. A pesticide must meet particular data requirements before it may be registered under the PMRA. At any time after registration, a CWQG may also be developed for the pesticide, with the method selected for development of the CWQG dependent on the toxicity data available for the pesticide. Although the data requirements for each process differ, this reflects two complementary processes of environmental management. This presentation will compare and contrast the guidance provided by each department and elaborate on the data requirements associated with each process.

Derivation of ideal performance standards for 10 pesticides in Canadian surface waters. E. Sabo[1], P. Jiapizian[1], M. Demers[1], K. Potter[1] and C. Mimeault[2] (PL)

[1]Environment Canada, Gatineau, QC; [2]University of Ottawa, Ottawa, ON

In 2006-07, Ideal Performance Standards (IPS) were developed for ten priority pesticides. These IPSs help fulfill the goal of the Canadian Agricultural Policy Framework to reduce impacts of agricultural activities to surface waters through the use of non-regulatory standards. The IPS standards specify the desired level of environmental quality in receiving waters affected by agricultural operations to protect biota. The method selected for IPS development is a tiered approach, where data availability determines which of the six methods is used to derive the IPS. Acute and chronic IPS were developed for each of the ten pesticides. Acute and chronic IPS were developed using the species sensitivity distribution (SSD) method for 2,4-D, endosulfan, and glyphosate. SSDs were used to derive the acute IPS for carbofuran, cypermetrin, diazinon, and flufenacet. The CCME (1991) process for interim Canadian Water Quality Guidelines was applied to develop chronic IPS guidance for these pesticides. The acute and chronic IPS for methamidophos and thifensulfuron-methyl were derived using the interim guideline process. We will describe the methods of IPS derivation and provide the IPS values for each pesticide.

Use of field evidence to calibrate pesticide aquatic risk models -- a step in the development of risk-based standards for the aquatic environment. ***T. Dawson[1], L. Singh[2], P. Mineau[1], M. Whiteside[1] and C. Morrison[1].***
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Risk assessments are an important tool in numerous fields, especially pesticide evaluation and registration. Our work through the National Agro-Environmental Standards Initiative (NAESI) project at Environment Canada has given us an opportunity to construct risk assessment models against field-documented impacts on aquatic communities. Sixty aquatic mesocosm and pond studies were assembled from the literature. Risk models were developed for several different taxa (e.g. crustaceans, insects, and algae). Species sensitivity distributions (SSD) were used to generate toxicity reference values and these were compared to single species LC50 values as predictors. Several physicochemical properties were also entered in the models and were found to be statistically significant predictors of population effects. We then obtained worst case water concentration values from a first tier runoff-drift model (USEPA GENEEC) for all agricultural pesticides in Canada and put these prospective concentrations through the best performing risk models. Work is currently underway with collaborators to determine more realistic environmental concentrations using advanced runoff modeling with a GIS interface. New concentration data along with our current risk assessment models will be turned into a risk-map to determine specific problem areas. With risk maps, problem areas can be isolated and geographically-based standards of good agricultural practice can be proposed.

Ecotoxicological assessment of sediments from a Canadian Forces firing range. ***P. Robidoux[1], V. Bérubé[1], C. Fournier, P. Bergeron[1], K. Kalomiris[1], C. Bastien[2], J. Hawari[1], B. Zajdlik[3], I. Young[4], H. Fanous[4] and G. Sunahara[1] (PL)***
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Some operational activities of the Canadian Forces (CF), such as firing practice and munitions testing, have led to the dispersion of energetic materials (EM) and metals in the Canadian environment. Sites contaminated by EM and metals may present a risk to humans and/or the ecological receptors. A CF Firing Range located on the St. Lawrence River, potentially contaminated by different EM (such as HMX, RDX and TNT) and metals resulting from the presence of unexploded explosive ordnances (UXO) and/or historical contamination, was recently investigated. The general objectives of the study were to: 1) assess the effect of sediments (and pore water) on selected receptors (e.g., microphytes, macrophytes, invertebrates) using rapid and/or sensitive toxicity tools (e.g., Microtox SP, SOS Chromotest, *L. minor* growth test, *Hyalella* survival growth or reproduction, *L. variegatus* bioaccumulation test); 2) develop and characterize (chemistry and toxicology) artificial sediments, based on CF Firing Range sediment characteristics (particles size, organic matter), to use as a reference sample for comparison with CF field

samples; 3) assess the effect of re-suspended sediments on selected receptors (e.g., Microtox, macrophyte *L. minor*, microphyte *Pseudokirchneriella subcapitata*) using elutriate samples; and 4) develop a bioaccumulation assay using freshwater mussel *(E. complanata)* and assess by exposure to selected sediment samples. This paper presents the approach, the selected toxicity tests and parameters of measurement as well as some key results.

A framework to estimate site-specific natural background guidelines in Canadian waters. J. Hill[1], S. Roe[1], B. Kilgour[2], N. Gautam[2], J. Perrone[2] and M. Davies[3] (PL)
[1]Environment Canada, Gatineau, QC; [2]Stantec Consulting Ltd., Ottawa, ON; [3]Coldwater Consulting, Ottawa, ON

Canadian Water Quality Guidelines (CWQGs) are voluntary tools that aim to protect all aquatic life stages during an indefinite exposure to harmful substances. However, it is not practical or generally desirable to set water quality goals beyond that which occurs naturally. While differentiating natural chemical concentrations and conditions from anthropogenic ones is challenging, a reasonable and coherent approach to background estimation is crucial to achieve more accurate reflections of water quality. Despite being a long-standing issue, information gaps still exist and clear direction is lacking. This presentation will detail and compare the potential approaches for estimating background in fresh-water bodies and illustrate the advantages and disadvantages through case studies. Four approaches that form the basis of the framework are (1) site-specific reference, (2) regional reference, (3) simple regional land-cover-based models, (4) complex physical engineering models, including those based on land cover, and those based on in-water processes. The application of any model or approach varies somewhat depending on the substance, the size and nature of the watercourse, and the underlying geology. The ultimate goal is to establish a nationally consistent but flexible framework for estimating natural background to inform the calculation of the Canadian Environmental Sustainability Indicators (CESI) Water Quality Indicator (WQI) program as well as the development of site-specific CWQGs

Engineering models to predict background suspended solids concentrations in rivers. N. Gautam[1], B. Kilgour[1], J. Hill[2], S. Roe[2], J. Perrone[1] and M. Davies[3] (PL)
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Rivers with naturally high suspended solids loads are often considered to have poor water quality (as determined by the Canadian Water Quality Index) because of associated high concentrations of adsorbed chemicals (nutrients, metals). Estimation of the natural background concentrations of suspended solids would be potentially useful, therefore, for "adjusting" water quality index scores. In this paper, we demonstrate how export coefficients, regional empirical models and more sophisticated physical models such as HSPF can be used to estimate background solids concentrations with cases studies. The

estimation of "background" concentrations generally involves forcing land use to a pre-development condition. Simpler models have relatively modest data requirements, but provide estimates with relatively broad confidence limits. The more complicated models are data hungry, but provide more reliable estimates of background suspended solids concentrations.

Assessment of the net effect of watershed alteration using measures of productivity. P. Stecko[1], C. Russel[2] and D. Berthelot[3] (PL)

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The Stanleigh Uranium Mine in Elliot Lake, Ontario, operated from 1958 to 1961 and from 1983 to 1996. During the second stage of operation, a sub-watershed was altered by damming eleven small lakes and constructing a series of spillways to provide sufficient water for milling operations. In 1996, as part of decommissioning, the mine wanted to restore the sub-watershed but was confronted with the problem that removal would result in a loss of fish habitat (i.e., lake sizes would be reduced with the removal of dams constructed for water diversion) and could potentially trigger substantial compensation requirements under the Fisheries Act. Rather than focusing on absolute changes in habitat dimensions, a productivity-based approach for achieving and demonstrating "no net loss" (NNL) of the productive capacity of fish habitat (the guiding principle of the Policy for the Management of Fish Habitat in Canada) was developed and applied. The approach involved documenting the changes in the surface areas of three key habitat types (lakes, wetlands and streams) that occurred with restoration and characterizing their natural productivity using multiple productivity measures. This presentation provides an overview of the regulatory framework for the alterations, the challenges associated with quantitative assessment of productivity, and key findings of the assessment.

Can clams define "real" ecological risks to help manage mine tailings? A "dark-side-of-the-moon" case study. M. McKernan[1], D. Huebert[1], J. L. Taylor[1], W. de Wit[1] and K. Mathers[1] (PL)

[1]Tetres Consultants Inc., Winnipeg, MB

Developing a practical long-term Tailings-Management Plan (TMP) for an acid-generating tailings area is a major challenge for any private-sector corporation or government. Finding ways to accurately define the current site-specific states of ecosystem health or ecosystem risk are important for defining and evaluating candidate components for a TMP that can measurably improve in ecosystem health. This is especially crucial in inaccessible northern locations where no working-mill revenue stream or community tax base is available to offset TMP costs.

Determining the current state of ecosystem health and current extent of environmental impacts depends upon accurate determinations of contaminant transport, mixing dynamics in a receiving watercourse, simultaneous measurements of water chemistry and biotic exposure, and correlation of tissue- and water-chemistry data. A two-year program

at an abandoned mine-site in a poorly accessible area of Northern Manitoba resulted in coupling of groundwater-contaminant-transport and river-mixing-zone models to identify the spatial extent of ecological risk. A biotic health-risk assessment using caged mussels, tissue-chemistry analyses, assessments of lethality and sub-lethal effects, and evaluations of water-column and river-sediment toxicity resulted in definition of site-specific Ecological Rick Thresholds for two metals of six present in discharges to the river. The mixing-zone model then predicted the spatial distribution of actual sub-lethal risk to sensitive biota in the river to 'benchmark' conditions before implementation of various site-management measures. The paper will outline approaches, results and implications for an emerging long-term TMP.

The tetrad approach: incorporating ecosystem function assessment into the sediment quality triad approach for contaminated site assessment in rivers. A. Gazeas[1], D. Baird[1] and S. Lambert[2] (PL)

[1]Environment Canada, Fredericton, NB; [2]Parks Canada, Ingonish Beach, NS

The Marrach is a small watershed draining the Cape Breton Highlands area of Nova Scotia. Over the past 15 years, a landfill has undergone major slumping of material into the adjacent Marrach Stream. Our study utilized a modified triad approach, integrating knowledge attained through study of (1) chemical analysis of water, sediment, and biota; (2) macroinvertebrate community structure; and (3) ecotoxicological testing with (4) the addition of measures of ecosystem function. This additional line of investigation involved the use of traditional leaf-litter bags to assess decomposition rates within the stream. Chemical analysis has identified a variety of contaminants in both stream sediments and groundwater. Sediment analysis found levels of lead, naphthalene and DDT exceeding guidelines by 71, 506 and 672% respectively. Groundwater analysis found the following metals exceeded guidelines by up to 2300%, including aluminum (1000%), copper (0.3%), iron (2366%), lead (100%) and zinc (366%). Surface water analysis found aluminum, copper and iron exceeded guidelines by 607%, 12.5% and 163% respectively. Benthic community surveys found evidence of impairment downstream of the landfill with a 79% decrease in sensitive EPT taxa abundance and a 63% reduction in total species diversity compared with the upstream sites. Sediment toxicity was evaluated via a 96-hour *in situ* feeding inhibition bioassay with the freshwater oligochaete worm *Lumbriculus variegatus*. Significant inhibition ($P<0.05$) was seen at two of three impacted sites with a 63% reduction in feeding. Leaf litter breakdown rates by the microbial community (aperture size of 350 µm) were similar between reference streams and declined significantly ($P<0.05$) at one of the impacted sites indicating some level of microbial impairment. The use of the modified triad showed that contamination had an adverse effect on aquatic life in Marrach Stream, at the community level (reduced diversity), at the level of individuals (feeding inhibition), and at the ecosystem function level (impairment of microbial detritus processing).

Bioavailability helps explain cause-effect relationships between contaminants-behaviour. K. Cheeseman[1], J. Hellou[1,2], E. Desnoyers[1], A. Grondlund[1], D. Johnston[1], J. Leonard[2], S. Marklevitz[1], A. Parsons[1], B. Robinson[1] and S. Robertson[1] (PL)

[1]Dalhousie University, Halifax, NS [2]Fisheries and Oceans Canada, Dartmouth, NS;

The reason why environmental studies attract attention is well illustrated by Mike Del Rizzo in the *Outdoor Canada* magazine of April 2006 that describes the dumping of "biozards" in a waterway where a fisherman has an accident, falls into the water and emerges bio-transformed into a mutant or perhaps a superhero. The comic strip leaves it up to the reader to imagine what type of follow up could take place in future episodes. Although this story is meant to make us smile, it should also make us stop and think in terms of underwater creatures experiencing the effects of urbanisation or witnessing the presence of substances that end up in the aquatic environment, a major sink of products used on a daily basis in our lives.

The path that led to investigating behavioural effects in amphipods and snails is based on determining the presence and uptake of contaminants by bivalves inhabiting Halifax Harbour. In order to respond to the priorities of the Department of Fisheries and Oceans, biological effects were included in the study of environmental quality that started by examining the level of priority pollutants in the inhabitants of that major harbour in the Atlantic provinces. Our initial intent was to determine if contaminants were bioavailable and bioaccumulated, and if this fate was associated with deleterious effects in the exposed biota. With time, our curiosity changed into developing a tool that could ultimately be used to prevent the detection or development of further chronic toxicity. Our field studies on the quality of Halifax Harbour formed the basis of our laboratory research on the behaviour of invertebrates exposed to contaminated harbour sediments receiving raw sewage effluents. Our field assessment covered the collection of water (Hellou *et al.*, 2004), sediments and mussels (Hellou *et al.*, 2000, 2002a and b, 2003, 2006, 2007; Robinson *et al.*, 2004), with the analysis of priority pollutants and newer chemicals of concern in these three matrices, along with potential biological effects in the bivalves. The concentrations of contaminants were compared to the Canadian Council of Ministers of the Environment (CCME) Sediment Quality Guidelines (SQG), where more than half of the sampled sites had polycyclic aromatic hydrocarbon (PAH) concentrations above the Probable Effects Level of toxicity (Hellou et al, 2002a). Specifically, 13 of 21 surface sediments collected around the 30 km of the harbour had PAH above the PEL threshold that is linked to a 50% probability of toxicity. The levels of these contaminants in sediments were also more than a hundred times higher than observed at reference sites, but within those described by other researchers for contaminated locations around the world (Table 1, Hellou et al, 2002a).

Table 1. Comparison of three PAH concentrations in sediments, especially harbours ($\mu g \cdot g^{-1}$,dry). PA is phenanthrene, FL is fluoranthene and PY is pyrene.

	Halifax	Saint John	Mediterranean	Boston	Sydney
PA	0.14-5.21	0.06-1.5	0.0003-7.77	0.05-63.7	1.11(1.34)
FL	0.08-6.11	0.06-1.2	0.0001-1.31	0.005-84.5	1.84(2.74)
PY	0.15-5.13	0.05-0.96	0.00009-1.15	0.20-66.8	1.53(2.32)

The concentrations of these lipophilic organic priority pollutants were as expected, much lower in seawater, by a factor of nearly 1,000,000 than detected in sediments, as can be compared in Tables 1 and 2 (Hellou et al, 2004). PAH were examined at three sites for a period of 18 months and tracked seasonal changes associated with the variable discharge of material coming from numerous point sources of contamination that include road runoff and that are affected by the amount of precipitation (Hellou et al, 2003, 2004b). The seawater from Halifax Harbour, as much the soluble as the particulate phase, was once again determined as being in the mid range of published results (Table 2).

Along with the abiotic sampling, inter-tidal mussels were collected, their particles' content depurated and examined for their body burden of various elements, 80 PAH, 159 polychlorinated biphenyls (PCB; King et al, 2003; Hellou et al, 2003), six DDT pesticides, coprostanol and/or musk fragrances (Gatermann et al, 1999; Helleur *et al.*, 2003; Hellou et al, 2003). The bioaccumulation results were examined in relation to a series of biomarkers reflecting the health status of this abundant organism. Assessment of the bivalves' health and body burden was conducted in three stages. An initial wide geographical coverage was performed on three occasions over a period of two years (Hellou et al, 2000 and 2002a and b). At first, basic measurements of health were performed and included lipid content and condition indices (CI). Our expectations regarding the effect of contamination was to detect lower CI in more contaminated animals, along with higher lipid content. However, since contamination was associated with the discharge of sewage effluents containing an abundance of food, the opposite was observed for the CI of more contaminated mussels. However, questions were raised about potential differences in the reproductive cycle of the animals that could explain different CI. This observation led to a more detailed temporal study to ascertain the interpretation of the impact of contamination on mussels' health.

In a second project, animals were collected frequently at three widely spaced sites chosen according to the earlier PAH bioaccumulation results to represent more, mid- and less-exposed bivalves (Hellou et al, 2003). The study of mussels' health was expanded by

Table 2. Comparing seawater concentrations of PAH ($ng \cdot L^{-1}$). PA is phenanthrene, FL is fluoranthene and PY is pyrene (Hellou et al, 2003).

Soluble phase				
PAH	Halifax	Iceland/N. Atlantic	Chesapeake Bay	Norway sewage effluents
PA	0.64-11.1	<0.005-0.03	2.2-4.1	72-1117
FL	1.1-5.7	<0.005-0.009	0.28-22.1	82-515
PY	0.41-2.9	<0.005-0.007	0.23-10.6	110-248

Particulate phase				
	Halifax	Baltic	Greece	Montréal sewage
PA	0.24-26.0	0.38-1.31	47-58	333/109 influent/effluent
FL	0.39-52.7	1.5-3.9	24-37	150/20
PY	0.30-43.5	0.30-43.5	27-50	138/23

examining sex ratio, gonad development, immune response and vitellogenin. The gonad development of the mussels examined during the summer also differed between these three sites. This more intensive sampling enabled us to conclude the validity of our interpretation of the simple biomarker results regarding lipid content and CI. The toxic effects at the three sites were rated as hypothesized from the PAH concentrations. A higher impact was linked to higher bioaccumulation of PAH, as well as Ag, Sn and coprostanol. Three of these chemicals are potentially sewage derived contaminants. Ag is used in photography; Sn has diverse "land" uses but is also an ingredient of anti-foulant paints, while coprostanol is a faecal marker. These additional results on bioaccumulation indicated that the proximity of numerous raw sewage effluents was most likely associated with the more impacted of the three studied sites in the central channel of Halifax Harbour.

A third follow up examined the extent of the "hot spot" by sampling mussels at a dozen sites located over a 6 km distance on either side of the more impacted and more studied downtown Halifax location (Hellou et al, 2006 and 2007). This investigation intended to determine if additional point sources around the harbour played a role in the discharge/leaching/detection of contaminants. The biological effects were expanded and still included chemical, biochemical, cellular, individual and population level indicators of health. While our studies were taking place, construction was ongoing in this dynamic harbour. A condominium and additional structures were built in the area of the waterfront that was previously sampled and found to display elevated levels of contaminants and the

more impacted mussels. During this last sampling, the analysed chemicals were uncoupled from one another relative to the studied effects. Of the more numerous and wider spread point sources covering the waterfront area of downtown Halifax, once again the presence of sewage played a key role in bioavailability and effects (Hellou et al, 2007). The sewage point source was now displaced 3km west, while the metalloids were more abundant 3km to the east, of the previous "hotspot".

Having assessed the health of abundant somewhat resilient organisms that can live in the water column and are exposed to water-soluble as well as particle-bound contaminants, it became obvious from the analysis of sediment that the quality of the latter needed investigation. Sediment sampling had demonstrated a lack of benthic fauna. Although the presence of sulphur was apparent along with the anoxic conditions, and these conditions would be detrimental to sediment' dwelling organisms, it was important to determine if the abundant chemicals associated with urbanisation and at levels above the PEL of SQG were bioavailable to potential aquatic inhabitants (Figure 1).

Two easily found and available small invertebrates representing a lower link in the food chain were collected from pristine inter-tidal beaches. Sediments were characterised for their organic carbon content and PAH levels. The amphipod *Corophium volutator* and mud snail, *Ilyanassa bsoluta* who prey on bacteria, diatoms and detritus and are the favourite prey of at least crabs, finfish, grey whales, seagulls and/or sand pipers were chosen as the subject of our investigations (Hellou et al, 2005; 2007 In Press; and Marklevitz In Preparation).

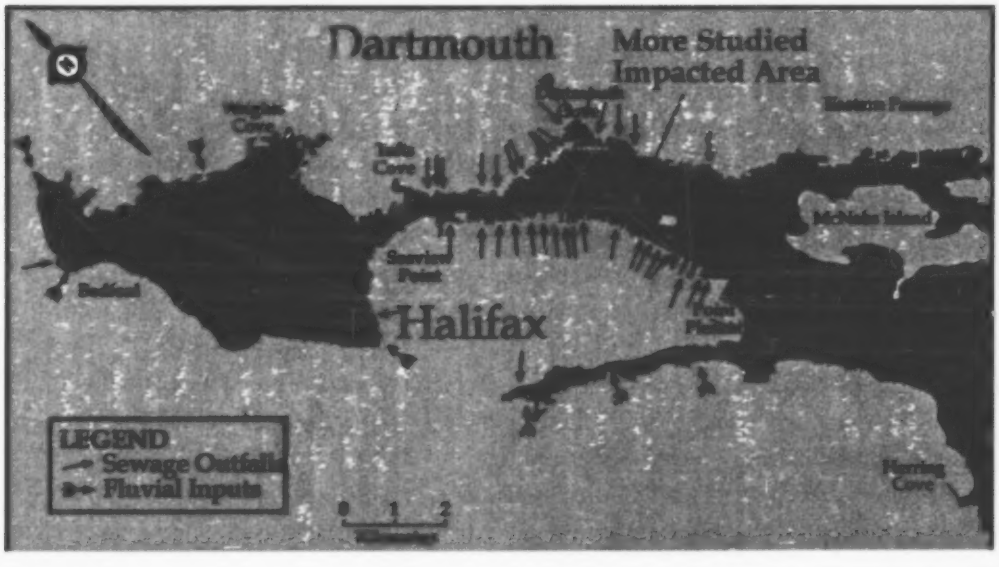

Figure 1. Map of Halifax Harbour with discharges of sewage outfalls and fluvial inputs, as illustrated by Buckley and Winters, 1993, indicating the more studied area of the harbour.

Our first experiments with amphipods involved standard toxicity tests, and survival was generally ideal. The bioavailability and bioaccumulation of PAH to amphipods was compared between exposures to harbour sediments and to PAH-spiked sediments (Hellou et al, 2007). This was the only fate pursued, since the biotransformation capacity of the animals was determined as below detection limits. The amphipods did not metabolise PAH added to reference sediments at concentrations equivalent to the highest levels measured in Halifax Harbour sediments. When these small crustaceans were placed on harbour sediments, they only accumulated a small fraction of the detected PAH as compared to spiked sediments and predicted by modelling using the equilibrium partitioning approach. In the pursuit of the environmental relevance of the observations regarding bioavailability, behavioural experiments were started using physical and chemical materials to better understand the habitat preferences of the amphipods. The addition of seaweed, sand, burned wood, and coal did not lead to a behavioural trend in terms of more or less avoidance of the spiked side of a tank with increasing amounts of physical disturbance (Hellou et al, 2005). However, for five harbour sediments collected in the central channel of Halifax harbour, an increased avoidance of contaminated and preference for reference sediments was observed with increasing amount of harbour added to reference sediments. The threshold for avoidance of contamination was associated with a level of PAH similar to the PEL of the CCME SQG (Hellou et al, 2005). The body burden of animals exposed to the threshold of avoided sediments was nearly a thousand times lower than that associated with narcosis. Comparing the level of PAH bioaccumulated by amphipods exposed to the avoided harbour sediments to that detected in amphipods exposed to PAH-spiked sediments leads us to conclude that from two to seven times more chemicals are likely implicated in the behavioural response (Hellou et al, 2007).

Studies were also initiated with mud snails and the important role of food as a confounding variable in the behavioural response was demonstrated (Marklevitz et al, In Preparation). A hungry animal will not behave as a well fed one. It will seek the food and will become susceptible to the potential and expected detrimental effects of the contaminated food through dietary exposure of material in sediments. The polarity of chemicals present in sediments and playing a role in behaviour was followed up using various solvent extractions and shown to be associated with non-polar chemicals, where PAH would be extracted along with many other types of natural products or synthetic organic contaminants such as chlorinated naphthalenes, fluorinated alkanes, phthalate esters, polybrominated diphenyl ethers and many more.

Our laboratory work was based on the parallel field research, i.e. observations performed on mussels exposed to particles that ultimately will deposit as sediments. If contaminants were bioavailable to the mussels, then it was assumed that a certain level of detrimental biological response "should" perhaps be observed. If the chosen biomarker in mussels was difficult to interpret, additional means of assessing the health of the animals were pursued.

In the case of the benthic environment, we chose small organisms abundant at pristine beaches to investigate the quality of the harbour habitat. To choose a short-term bioindicator of sediment quality, we stepped into the organisms shoes and wondered how we would behave when facing a contaminated environment. Behaviour was deemed a cumulative response that would integrate across the senses of smell, taste, (eating, drinking), view, touch, and that perhaps could be summarised as "feelings." Changes in behaviour could represent a defence mechanism, an innate reflex based on a preservation instinct. Chemistry provided a unique tool with a quantitative aspect used to address the question of cause-effect relationships. Bioavailability enabled the interpreting biological effects. The construction of three sewage treatment plants is ongoing in the Halifax Regional Municipality, and these will make a difference.

Our studies were conducted to understand environmental interactions and also to develop a green approach that could be used to prevent expected (according to the PEL of the SQG) deleterious effects under longer exposure. Our work pursued answers by addressing government priorities. It provided an in-depth accurate view of the state of the environment and placed the results in an international, balanced perspective using an integrated, tiered approach to assess an ecosystems' health. The pursuit of more work should be encouraged and funded. Discovering the state of the environment enables society to make choices to enhance the habitat of future generations.

Acknowledgements

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Proposed amendments to Environmental Emergency Regulations under Section 200 of CEPA 1999. K. Hradecky[1] and K. Ketcheson[1] (PL)

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Abstract

On November 18, 2003 the Environmental Emergency (E2) Regulations came into force (Government of Canada, 2003) under the authorities of section 200 of the *Canadian Environmental Protection Act (CEPA), 1999* (Government of Canada, 1999). These regulations require the development and implementation of environmental emergency plans for 174 substances with associated thresholds that, if released to the environment as a result of an environmental emergency, may harm human health or environmental quality. Companies in Canada storing these substances, with the conditions stipulated in

section 200, will need to have their E2 plans implemented within one year of the regulations coming into force.
This presentation will review the proposed amendments to the regulations on Canadians. Presently, a total of thirty-four new substances are being recommended for addition to the regulations based on the analysis of the substances listed in the Toxic Substances List (http://www.ec.gc.ca/ceparegistry/subs_list/Toxicupdate.cfm). Thresholds will be explained and their associated concentrations.

Introduction

CEPA, 1999 provides the Government of Canada instruments including regulations to protect the environment and human health. CEPA 1999 Part 3, Section 44, authorizes the Minister of the Environment to collect and publish data on environmental quality in Canada, conduct research and studies on pollution control and environmental contamination, and on the quality and state of the Canadian Environment. As a result of this authority under CEPA 1999, Environment Canada has compiled a list of substances that have been found to be contaminants within Canada's ecosystems and have been known to cause harm to the environment and/or humans. In this manner, these substances are referred to as "toxic" under CEPA 1999 and are recommended for addition to the List of Toxic Substances (Schedule 1) of the Act.
The preparation of environmental emergency plans can be required for substances that have been assessed to be "toxic" under CEPA 1999 and are on the List of Toxic Substances or are recommended for addition to that list. CEPA clearly outlines this authority in Part 8 under Section 199 (CEPA, 1999). This list can be located on the web, under the title "Toxic Substances List" (http://www.ec.gc.ca/ceparegistry/subs_list/Toxicupdate.cfm). This Toxic Substances List has some 85 substances as of December 27, 2006. For the entire listing of the substances that were studied see Ketcheson and Shrives, 2004.
It is important to understand that the origin of these substances was the result of extensive examination of their respective concentrations in the environment, whether they were located in water, land or air. Once the quantity of substance was detected in a certain media, it was then a matter of determining if the concentration of that substance could theoretically cause a toxic effect to an organism. This determination was undertaken by comparing the measured in situ concentration to a known in vivo experiment. If the substance was found to cross a certain toxicity threshold then the substance was referred to as a "CEPA-toxic", and was subsequently added to Schedule 1.
The substances listed in Schedule 1 were assembled so as to highlight those that would require a mechanism to minimise, or virtually eliminate, their exposure to the environment. Typically, the majority of the substances fall under the umbrella of E2 plans or pollution prevention (P2) plans. E2 plans cover those substances that could enter the environment via a catastrophic spill, whereas P2 plans address those substances that are released via emissions, effluents, or chronic releases.

This method of protecting Canadians is useful, but there are some situations where the devised system has not been effective. Phenol for example is a substance that was examined and was not recommended to be placed on Schedule 1. Phenol was not listed in Schedule 1 because the substance degrades in the environment and thus it was not found in sufficient quantities to cause a harmful effect on the environment. However, from an emergency perspective, there has been a case where a man had phenol spilled on his back, and he died 10 minutes later (HSDB, 2005). Fortunately, Section 200 of CEPA allows substances to be added to the E2 regulations if they are found to be hazardous and does not require them to be labelled as a "CEPA-toxic".

On the other hand, road salts have been found to be toxic to the environment. If one were to spill road salts, there would be virtually no emergency pathway for this substance. One only requires shoveling the substance back into the container, with essentially no concern for toxic effects. Thus, no emergency plan would be required for road salts. But road salts were found to persist in the environment and are causing toxic effects to organisms and/or plants. Hence by this method of analysis, special provisions have been created for road salts by calling for a code of practice for the dispersing of this substance on the roads. Here, P2 plans were applicable instead of E2 plans.

This set of amendments has addressed the question of which of the substances on Schedule 1 require environmental emergency plans. Although Schedule 1 substances are continually being added, these sets of amendments have brought to closure many of the substances that had been on Schedule 1 since the 1990's.

Because the current regulations did not take into account possible effects to the environment, this first set of amendments, addressing only substances on Schedule 1, has included environmental thresholds. The current regulations will be re-assessed for their potential to cause damage to the environment at a later time.

Some of the substances are not found on Schedule 1 but they belong to classes of substances. For example, sulphur hexafluoride is within the class of substances called "Inorganic Fluorides". The cadmium compounds are under the heading "Inorganic Cadmium Compounds". Naphthalene can be located in the class called "Polycyclic Aromatic Hydrocarbons". Nickels are found in "Oxidic, Sulphidic and Soluble Inorganic Nickel Compounds", and chromium substances are in "Hexavalent Chromium Compounds". Of the 65 substances originally listed on Schedule 1, there are actually 97 reports because the classes of substances can have many compounds included.

Three of the substances, namely styrene, acetic acid and ammonium nitrate, were never on Schedule 1 but industry requested them to be added to this set of amendments because they were chemicals of concern. This illustrates that industry is aware of the potential hazards of catastrophic spills of certain substances and is demonstrating a proactive approach to protecting their employees and the population at large.

Of the 65 substances and classes of substances listed, some were rejected because they were regulated under another Act of Parliament. Others were not accepted for E2 plans due to the fact that they were by-products or contaminants and were not stored. A few

did not require E2 plans because they were being phased out and were no longer allowed to be manufactured. For others, P2 plans were more applicable than E2 plans. The remaining substances—a total of 34, with one being deferred due to lack of scientific data—were assigned threshold quantities and are being proposed for addition.

The Current List of Regulated Substances

The E2 regulations currently have 174 substances and their associated thresholds in the Canada Gazette II notice registered 20 August, 2003 (Government of Canada, 2003). These substances have been divided into two categories, Part 1 (flammable substances) and Part 2 (other hazardous substances). In order to know how their respective thresholds were calculated see *Ketcheson and Shrives* (2005).

The Proposed List of Substances

This list of substances was first mentioned in *Ketcheson and Shrives* (2004); since then there have been 97 reports written on these substances or classes of substances that are available on request (see e-mail in title). The list of proposed substances for addition to the E2 regulations is shown in Tables 1, 2 and 3.

Table 1 is for the flammable substances and substances that can behave as explosives under certain circumstances. Both of the substances below acquired their threshold quantity due to their reactivity. Styrene's threshold was set at 4.5 tonnes because of its potential to BLEVE (boiling liquid expanding vapour explosion), while ammonium nitrate's threshold was set at 20 tonnes due to its capability to explode in the solid form.

Table 1: Proposed for Addition to Part 1 of Schedule 1

CAS Number	Name of Substance	Threshold Quantity (Tonnes)	Concentration (%)
100-42-5	Styrene	4.5	10
6484-52-2	Ammonium nitrate (in liquid form)	20	81
6484-52-2	Ammonium nitrate (in solid form)	20	60

Table 2 shows those substances that possess at least 10 mm Hg vapour pressure and can cause a toxic inhalation plume. These thresholds were determined using the Risk Management Process from the United States Environmental Protection Agency (US EPA). The formula for the calculation can be found in *Ketcheson and Shrives* (2005a).

Table 2: Proposed for Addition to Part 2 of Schedule 1

CAS Number	Name of Substance	Threshold Quantity (Tonnes)	Concentration (%)
64-19-7	Acetic acid	6.8	95
75-09-2	Dichloromethane	9.1	1
2551-62-4	Sulphur hexafluoride	9.1	10

Table 3 is a compilation of substances that may be a human and/or animal carcinogen, and/or are toxic to fish. Some of the substances may have more than one Chemical Abstract Service Number (CAS Number) due to hydrates. All of the substances below have less than 10 mmHg vapour pressure and, hence, cannot be associated with Part 2. Although 34 substances are being proposed for addition to the E2 Regulations, the list is not final since negotiations are still underway. Therefore the list may be modified before it is published in Canada Gazette Part 2.

Table 3: Proposed for Addition to Part 3 of Schedule 1

CAS Number	Name of Substance	Threshold Quantity (Tonnes)	Concentration (%)
56-23-5	Tetrachloromethane (Carbon tetrachloride)	0.22	1
79-01-6	Trichloroethylene	1.13	1
91-20-3	Naphthalene (in liquid form)	4.5	10
91-94-1	3,3'-dichlorobenzidine	1.13	1
117-81-7	Bis(2-ethylhexyl) phthalate (DEHP)	0.22	1
127-18-4	Tetrachloroethylene (Perchloroethylene, Perc)	1.13	1
373-02-4	Nickel acetate	0.22	10
1303-28-2	Arsenic pentoxide	0.22	10
1306-19-0	Cadmium oxide	0.22	10
1306-23-6	Cadmium sulphide (Cadmium sulfide)	0.22	10
1313-99-1	Nickel oxide	0.22	10
1327-53-3	Arsenic trioxide (Arsenic(III) oxide)	0.22	10

CAS Number	Name of Substance	Threshold Quantity (Tonnes)	Concentration (%)
3333-67-3	Nickel carbonate	0.22	10
7440-38-2	Arsenic	0.22	10
7718-54-9	Nickel chloride	0.22	10
7775-11-3	Sodium chromate	0.22	10
7778-39-4	Arsenic acid	0.22	10
7778-43-0 & 10048-95-0	Sodium arsenate	0.22	10
7784-46-5	Sodium arsenite	0.22	10
7786-81-4 & 10101-97-0	Nickel sulphate (Nickel sulfate)	0.22	10
7789-00-6	Potassium chromate	0.22	10
7738-94-5 & 1333-82-0	Chromic acid (Chromium trioxide)	0.22	10
10108-64-2	Cadmium chloride	0.22	10
10124-36-4	Cadmium sulphate (Cadmium sulfate)	0.22	10
10588-01-9	Sodium dichromate	0.22	10
13138-45-9 ; 13478-00-7	Nickel nitrate	0.22	10
15699-18-0	Nickel ammonium sulphate (Nickel ammonium sulfate)	0.22	10
81741-28-8	Tributyl tetradecyl phosphonium chloride (TTPC)	0.22	10
25154-52-3; 104-40-5 & 84852-15-3	Nonylphenol	1.13	10

Conclusion

Environment Canada has created environmental threshold quantities in order to fulfill the requirements of CEPA, which is to protect humans and the environment. Since the current set of regulations only took into account potential human impacts as a result of spills, the amendments provide additional considerations for potential spills to the environment with these sets of substances. Hence, at some point in the future, all of the current substances will be re-examined to determine their possible impacts to the environment in the event of a spill.

Establishing threshold quantities for aquatic life can be difficult, especially when some chemicals can affect aquatic species to varying degrees, when conditions such as pH, temperature, and hardness of water are constantly changing. This methodology provides a means of demonstrating that those substances capable of causing harm to fish, with bioaccumulation and persistence criteria included, will have lower threshold quantities assigned.

Some of the substances, such as tetrachloroethylene, may have more than one threshold quantity assigned. This substance had the following thresholds: 1.13 tonnes for aquatic, and 6.8 tonnes for air inhalation toxicity. The amendments will state the most stringent threshold quantity, which in this case is 1.13 tonnes. Due to the danger of the substance entering the water and the air environments, it is expected that the E2 plan would cover both such possibilities.

The proposed regulations were published in Canada Gazette I on June 9th, 2007, and it is expected that the substances will be published Canada Gazette II for the winter of 2008. Consultations have already taken place with key stakeholders and industry, but the amendments have not formally been accepted or reviewed by the public at large. Thus, there may be unanticipated changes to the amendments in the near future after the consultation period has passed.

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Selection of aquatic toxicity criteria for Environmental Emergency (E2) Planning. K. Hradecky[1] and K. Ketcheson[1] (PL)

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Abstract

The Environmental Emergency Regulations of section 200 in Part 8 of the *Canadian Environmental Protection Act* (CEPA, 1999) require environmental emergency (E2) plans for chemicals based on criteria encompassing three hazard rating sections: environmental, human, and physical properties. Within the environmental hazard rating is a means to determine if a company is recommended to implement an E2 plan for their substance. If the aquatic toxicity value for a substance is less than or equal to 100 $mg \cdot L^{-1}$ for a 96-hour LC_{50} fish test, the substance is recommended for an E2 plan. Depending upon the results of the test, if the substance is classified as very toxic, then a lower threshold quantity for the E2 plan is recommended. The threshold quantity could be further reduced based on the persistence and bioaccumulation values The aim of this paper is to explain the rationale behind the selection of the aquatic toxicity criteria, within the environmental hazard rating, for Environmental Emergency Plans. This paper will examine different international and regional aquatic toxicity criteria and the rationalization for choosing the E2 aquatic toxicity criteria.

Introduction

The Environmental Emergency (E2) Regulations were published in Canada Gazette Part 2 on September 10, 2003 and came into force on November 18, 2003 (Government of Canada, 2003). The Regulations apply to anyone using or storing any of the 174 regulated substances. An additional 34 substances are being proposed for addition to the E2 Regulations based on the criteria from the Environmental Emergency Risk Evaluation Framework (REF).

The REF is a rating system done in Microsoft Excel that incorporates the CRAIM criteria for human health and safety. There are three sections used to evaluate a substance: environmental hazard, human hazard, and physical hazard. Each section is sub-divided into categories. Around the world, the cornerstones of most chemical hazard evaluations are persistence, bioaccumulation and toxicity (PBT) criteria. In Canada, the Toxic Substances Management Policy (TSMP) of CEPA uses PBT criteria for determining when an organic chemical is to be considered for "virtual elimination" in Canada. This paper will focus on three categories found within the environmental hazards section: persistence, bioaccumulation and aquatic toxicity.

Persistence

Chemical substances that degrade slowly in the environment (i.e., are relatively resistant to biodegradation, hydrolysis and photolysis processes) are classified as persistent and represent potential environmental problems. Persistence is measured as a half-life, the time needed to reduce the quantity of a chemical by transformation to half its initial quantity in the environment. A compound released into the environment has a tendency

to partition (i.e., accumulate) into one medium (air, water, soil or sediment) more than another. Partitioning, transport and transformation rates differ in each medium.
The persistence definition according to the Canadian Environmental Protection Act 1999 (CEPA, Government of Canada, 1999) is:

Air:	i) its half-life $\geq$ 2 days
	ii) it is subject to atmospheric transport from its source to a remote area
Water/Soil:	its half-life $\geq$ 182 days
Sediment:	its half-life $\geq$ 365 days

Another important factor which is also considered is the bioaccumulation potential of a substance.

Bioaccumulaton

Bioaccumulation is the process of a chemical moving from the medium surrounding an organism (water, sediment, soil or air) or the diet into the organism from all possible exposure routes and is expressed as a bioaccumulation factor (BAF). Non-dietary bioaccumulation in aquatic organisms is referred to as bioconcentration factor (BCF). It is the process of a chemical moving from water to an organism, and only water is considered as the exposure medium.
The frequency distributions of log Kow and bioaccumulation factor (BCF) were calculated from empirical data on BCF and log Kow obtained from 745 chemicals from the Syracuse Research Centre. The distributions were examined to determine if bioaccumulation criteria should include both types of measurements. Generally, BCF data are preferred over Log Kow, but Log Kow are easier to obtain empirically. According to the study, a range of Log Kow $\geq$ 3 appeared to be the most precautionary approach to capture chemicals. However, a range of Log Kow $\geq$ 3 would have caught 36% of the chemicals when most agencies cited Log Kow $\geq$ 4. After examining the data it was decided to use the virtual elimination data from *CEPA 1999* as an extreme hazard, BCF $\geq$ 5000 or Log Kow $\geq$ 5, and incorporate the Organization for Economic Cooperation and Development (OECD)/ Joint Group of Experts on the Scientific Aspects of Marine Environment Protection (GESAMP) criteria as a very high hazard (BCF $\geq$ 500 to 5000 or Log Kow $\geq$ 4 to < 5 (unless BCF < 500). It was concluded that Log Kow $\geq$ 3 was too low, hence not appropriate.
The next section will explain the selection of the aquatic toxicity thresholds used in E2 Regulations.

Aquatic Thresholds

Acute toxicity is determined using primarily a fish 96 hour LC_{50}. Scoring for acute toxicity of aquatic species is based on the classification table from the Organization for Economic Cooperation and Development (OECD, 2001). In addition, a robust study evaluation will determine which value will be used for classification. The most sensitive value for a freshwater fish will be selected for the robust study. If the value fails the

robust study, then the next most sensitive species will be selected. Only data on Canadian species are used.

Table 1 shows the fish toxicity criteria table with its corresponding scale. Values for 96 hour LC_{50} fish toxicity data that are less than or equal to 100 $mg \cdot L^{-1}$ may require an E2 plan. Any value over 100 $mg \cdot L^{-1}$ is classified as practically non-toxic and was not included for environmental thresholds.

Table 1: Acute Toxicity 96 Hrs LC_{50} Rating for Aquatic Species (OECD, 2001)

Category	Aquatic Toxicity ($mg \cdot L^{-1}$)
Extremely Toxic	≤ 0.1
Highly Toxic	> 0.1 to ≤ 1
Moderately Toxic	>1 to ≤ 10
Slightly Toxic	>10 to ≤ 100
Practically Non-Toxic	>100

The assessment of the aquatic toxicity threshold will also include data from persistence and bioaccumulation, when available. The persistence and bioaccumulation criteria have been selected and can be seen in Table 2 below. CEPA has classified the virtual elimination criteria for the persistence of a substance in sediment to be greater than or equal to one year. Since the water persistence for CEPA virtual elimination is greater than or equal to six months, then the water criteria was selected over the sediment value. When the persistence, bioaccumulation, fish toxicity, and thresholds are placed together, they result in table 2, as shown below. The first step in using the table is to locate where the value for the most sensitive fish is located within the Acute Aquatic Toxicity row. If there is no available information on persistence (P) or bioaccumulation (B), then the fish toxicity value is related to the threshold quantity, which is the bottom row. If P and B criteria exist, then the acute aquatic toxicity defaults to the lower column of the P or B values with the corresponding drop in threshold quantity. If the P and B criteria are higher than the fish toxicity, then the lower threshold quantity is maintained. For example, suppose the acute aquatic toxicity is 15 $mg \cdot L^{-1}$. This value corresponds to a threshold quantity of 9.1 tonnes. Other data on the same substance suggests that the P value is 8 months and the BCF is 750. Thus, the acute aquatic toxicity defaults to 0.22 tonnes because the P value falls within the classification of an extreme hazard. The BCF criteria was only classified as a very high hazard, thus it is not the dominant criteria for the acute aquatic toxicity.

Because of this system, any substance that can remain in water for long periods of time will continue to contribute to the aquatic toxicity. Also, if a substance is bioaccumulative in water, then the substance may accumulate in the organism and may be transported along the higher trophic levels of the food chain. Due to the inherent hazardous potential

of substances that are persistent and bioaccumulative, these parameters have been taken into account along with the acute aquatic toxicity information.
The threshold quantities for the aquatic toxicity were taken from the Risk Management Process (RMP) quantities used for the vapour cloud explosion potential partially shown in table 3, (J.P. Lacoursière Inc., 2002). The most hazardous of the aquatic toxicity was assigned a value of 0.22 tonnes. The largest fish toxicity parameter was given the highest threshold quantity of 9.10 tonnes. The rest of the values between 0.1 and 100 $mg \cdot L^{-1}$ were evenly staggered between the threshold ranges.

Table 2: Acute Aquatic Toxicity Thresholds

Criteria	Extremely Toxic	Highly Toxic	Moderately Toxic	Slightly Toxic
Persistence (water)	≥6 months	≥ 2 months to < 6 months	N/A	N/A
Bioaccumulation	BCF ≥ 5000 or Log Kow ≥ 5	BCF ≥ 500 to < 5000 or Log Kow ≥ 4 to < 5 (unless BCF < 500)	N/A	N/A
Acute Aquatic Toxicity (96 hrs LC_{50} – $mg \cdot L^{-1}$)	≤ 0.1	> 0.1 to ≤ 1	> 1 to ≤ 10	> 10 to ≤ 100
Threshold Quantity (Tonnes – lbs)	0.22 (500 lbs)	1.13 (2 500 lbs)	4.50 (10 000 lbs)	9.10 (20 000 lbs)

Table 3: Threshold Values Index

Fish Toxicity ($mg \cdot L^{-1}$)	Threshold Quantities metric tonnes (lbs)
≤ 0.1	0.22 (500)
--	0.45 (1 000)
> 0.1 to ≤ 1	1.13 (2 500)
--	2.27 (5 000)
> 1 to ≤ 10	4.50 (10 000)
--	6.80 (15 000)
> 10 to ≤ 100	9.10 (20 000)

Since groundwater, drainages, tributaries and rivers can all lead to contamination of a water body, it is proposed that these acute toxicity thresholds would apply even if a fixed

facility did not have any visible water body within the boundaries of the company's location.

The following section provides a brief explanation for dealing with mixtures of substances found on the E2 Regulations.

Mixtures

The current regulations and the amendments show a threshold quantity that is given as 100% purity. Mixtures for substances listed in the regulations under Parts 1 and 2 already have methods to determine what the threshold quantity would be for mixtures. However, for the substances in Part 2b it was decided to divide the threshold quantity by the percent purity. This process is familiar to industry since it is already used to determine the threshold quantity for toxics. For example, if the threshold quantity for a certain substance in the E2 Regulations is 1.13 tonnes, and the percentage purity is 36%, then the new threshold quantity would be:

$$\frac{1.13}{0.36} = 3.14 \; tonnes$$

This is the standard approach, but there is nothing preventing a company from using the aquatic toxicity guideline written by Environment Canada. This guideline has been written with Canadian and OECD guidelines in mind. Thus, the guideline has been harmonized between countries to give a standardized scientific approach to determining the 96 h LC_{50} for aquatic species. The guideline is called, "*Guidance Document on Statistical Methods for Environmental Toxicity Tests (EPS 1/RM/46-March 2005)*", and another useful document is called, "*Guidance Document on Application and Interpretation of Single-species Tests in Environmental Toxicology (EPS 1/RM/34-1999)*". In this manner, instead of estimating the toxicity level, one could experimentally determine the LC_{50} value with a credited laboratory for a mixture, and then subsequently determine the threshold quantity, instead of doing a calculation. The calculation method is an acceptable method to deduce the new threshold quantity.

Conclusion

Environmental threshold quantities were created by Environment Canada in order to fulfill the requirements of CEPA, which is to protect humans and the environment. The environmental hazard ratings consist of persistence, bioaccumulation, and aquatic toxicity. These criteria enable one to understand the impact each substance would have on the environment or humans if it were involved in an environmental emergency situation. The criteria chosen for aquatic toxicity is consistent with the OECD classification table thereby creating international harmony.

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Risk assessment priorities and approaches for metal-containing substances. M. Eggleton[1], Y. Couillard[1], J. Gauthier[1], R. Goulet[1], A. Gosselin[1], O. Marois[1] and P. Doyle[1] (PL)

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Under the Canadian Environmental Protection Act of 1999, the Ministers of Environment and Health had to categorize, based on human health and ecological considerations, all 23,000 substances on the Domestic Substances List (DSL) by September 2006. This categorization exercise identified approximately 4,300 substances that are considered priorities for assessment, including approximately 1100 metal-containing substances. A priority-setting exercise is underway to establish a post-categorization plan for all of these substances—i.e., which substances will be assessed first, which have critical data gaps that need to be filled prior to assessment, and which are likely of lower concern. Certain metal-containing substances have already been selected for assessment as part of a challenge for high-concern priorities in the context of the Government of Canada's Chemicals Management Plan. Some factors being considered in priority setting will include (but not be limited to) existing international data-gathering activities, current management practices limiting exposures, current commercial status in Canada and complexity of the hazard or exposure potential of the substances. Further approaches are being considered for the assessment of metal-containing substances, including grouping substances by a common moiety of concern. These approaches and priorities will be discussed in this presentation.

"Below-MDL" values: What are they and how should datasets including them be treated? R. Green[1], J. Kennish[1], J. French[1] and J. Banta[1] (PL)

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Prince William Sound Regional Citizens' Advisory Council (PWSRCAC) conducts a long-term environmental monitoring program mandated by the US Oil Pollution Act of 1990. The analytical data from from this study are often above instrumental detection limits but near or below the method detection limits (MDL). Other studies suggest the

possible environmental relevance of PAH contaminants at these levels. This has prompted PWSRCAC to consider the use of "below-MDL" data in both univariate analysis and multivariate pattern recognition. These considerations include what value should be used in place of "below-MDL" for calculation of means and other summary statistics and for doing hypothesis-testing univariate statistics—e.g., t-tests, ANOVA? Are descriptive statistics, e.g. pattern recognition by multivariate analysis, valid with data which include "below-MDL" values? Might this lead to misinterpretation of low concentration PAH frequencies in a pattern analysis? How is such a multivariate analysis best done? What is "standard practice" regarding all of this, for the US and Canada? What has been suggested by statisticians that has not (yet) been adopted as standard practice?

Effects of in-stream pipeline crossing construction on aquatic ecosystems and methodologies for impact assessment. L. Levesque[1] and M. Dubé[2] (PL)

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Pipeline crossing construction typically alters river and stream channels, potentially causing detrimental effects on aquatic ecosystems. A review was conducted to examine documented effects of crossing construction on fish and fish habitat in rivers and streams, and thereby recommend an approach for monitoring and assessment of impacts associated with this activity. Pipeline crossing construction was shown to compromise not only the physical and chemical nature of fish habitat, which are conventional components of effects assessments for pipeline crossing construction, but also the biological habitat as well as fish behavior and physiology. Indicators of effect included: water quality (total suspended solids), physical habitat (particle size, channel morphology), benthic invertebrate community structure and drift (abundance, species composition, diversity, standing crop), and fish behavior and physiology (hierarchy, feeding, respiration rate, loss of equilibrium, blood hematocrit and leukocrit levels, heart rate and stroke volume). The Before-After-Control-Impact approach used in Environmental Effects Monitoring was recommended for use in Environmental Impact Assessments for pipeline crossings of water bodies.

Environmental fate of phthalate di-esters and mono-esters in sediment and organisms of an aquatic food web. M. McConnell[1], F. A. Gobas[1], J. D. Blair[2] and M. G. Ikonomou[2] (PL)

[1]Simon Fraser University, Burnaby, BC; [2]Dept. of Fisheries and Oceans, Sidney, BC

Phthalate di-esters (DPEs) are a family of industrial chemicals used in a wide variety of consumer products, mostly as additives to impart flexibility in plastic materials. The biodegradation of DPEs in the environment results in the formation of phthalate mono-esters (MPEs) as intermediate hydrolysis products. Very little is known about the distribution, abundance, behavior, fate and toxicity of MPEs in the environment, yet such information is important for the evaluation of the environmental impact of DPEs. The persistence (P), bioaccumulation potential (B), and inherent toxicity (iT) of phthalate

esters (PEs) are important regulatory criteria. The Canadian Environmental Protection Act (1999) has focused on P, B, and iT of commercially used PEs as well as many other industrial substances. This paper reports on the P and B for DPEs and MPEs, with the goal of correctly assessing DPEs under CEPA. Specifically, we report on the (1) distribution and bioaccumulation potential of DPEs in organisms of a marine food-web, (2) metabolic transformation of DPEs in marine fish species, (3) biodegradation of DPEs in natural marine sediments, (4) distribution and bioaccumulation potential of MPEs in organisms of a marine food-web, and (5) biodegradation and sorption of MPEs in marine and freshwater sediments. We conclude that although environmental concentrations of DPEs are 10-1000 times greater than PCB concentrations in the aquatic food web, (1) the lipid normalized concentrations of DPEs do not vary with trophic level, (2) DPEs are rapidly hydrolyzed in the gastro-intestinal tract of the fish and MPEs are detected in the fish tissues, (3) MPEs do not biomagnify in the aquatic food web, and (4) MPEs do not appear to be persistent substances in natural marine or freshwater sediments. The toxicity of phthalate esters is not discussed here.

Atlantic Eco-RBCA: Development of ecological health-based soil, sediment and aquatic criteria for petroleum hydrocarbons. ***R. Mroz[1], K. Doe[2], U. Klee[3], P. Miasek[4], M. Stephenson[5] and R. Willis[6] (PO)***

[1]Environment Canada, Dartmouth, NS; [2]Environment Canada, Moncton, NB; [3]Dillon, Cambridge, ON; [4]Imperial Oil, Toronto, ON; [5]Jacques Whitford Ltd, Fredericton, NB; [6]Intrinsik Environmental Sciences Inc., Halifax, NS

A new "Eco-RBCA" Task Group has been created by the Atlantic Partnership in RBCA Implementation (Atlantic PIRI) to update and enhance the current ecological screening process in the Atlantic RBCA (Risk-Based Corrective Action) Toolkit. This Task Group brings together experts in the field of ecological risk assessment to specifically address the ecological risks posed by petroleum hydrocarbons. The phased work plan includes an updated ecological screening checklist, and the development of both soil eco-contact criteria and aquatic screening criteria, based upon existing literature and the use/application of the Petro-tox model (CONCAWE). Significantly, the Task Group also proposes to develop sediment quality criteria for petroleum hydrocarbons, based on new toxicity testing that is planned to occur in early 2008. As the various phases are completed by the Task Group, there will be amendments to the Atlantic RBCA User Guidance in order to ensure effective, consistent application of these new tools. Atlantic PIRI is a regional multi-stakeholder group that focuses on contaminated sites in Atlantic Canada. The work of this Task Group reflects Atlantic PIRI's commitment to continuous improvement.

Evaluation of the Screening-Level Concentration (SLC) approach for deriving sediment quality guidelines for uranium mines. C. Burnett[1] and K. Liber[1] (PO)
[1]University of Saskatchewan, Saskatoon, SK

Sediment quality values can be derived using different approaches and are used in environmental management, regulations, reclamation and risk assessment. The Screening-Level Concentration (SLC) approach is used in Ontario to derive lowest-effect levels (LEL) and severe-effect levels (SEL) based on benthic communities indices (abundance and richness) and total contaminant concentrations in sediment. These contaminant levels are used as sediment quality guidelines. The SLC approach has also been adopted by the Canadian Nuclear Safety Commission (CNSC) to set guidelines for metals (As, Cr, Cu, Pb, Mo, Ni, Se, U, V) and radionuclides (226Ra, 210Pb, 210Po) for use at northern Saskatchewan uranium operations. To evaluate the appropriateness of this approach, historical sediment and benthic community data from northern Saskatchewan uranium operations were collected and examined. First, data was explored to find sites (>50 in total) that had paired data (benthic community measurements and metal/radionuclide concentrations in sediment sampled approximately on the same day). Secondly, sediment chemistry data was screened for exceedences of LEL and SEL values based on average concentrations at each site. Lastly, for sites where guidelines were exceeded, a summary of reported effects on the benthic community (statistically different from a reference site, or a >20% decrease in abundance or richness compared to a reference site) was recorded. A summary of the results of this analysis are presented and discussed. The findings from this evaluation highlight where improvements to the SLC approach are needed.

Suspension of non-functionalized multi-walled carbon nanotubes (CNTs) in freshwaters: Examining the effects of CNT diameter and dissolved organic matter. N. O'Driscoll[1], T. Messier[1], M. Robertson[1] and J. Murimboh[1]
[1]Acadia University, Wolfville, NS

Abstract

Global demand for CNTs is expected to reach $200 million in 2009, and will likely surpass $9 billion by 2020. As CNTs become commonplace, the range of uses is expected to expand dramatically as will the potential for release into ecosystems. Recent research suggests that CNTs display increased dispersion in the presence of dissolved organic matter, thus increasing their ability for transport. However, it remains unclear how CNT characteristics and dissolved organic matter (DOM) structure will affect the dispersion of CNTs in a natural environment. Our research aims to close this knowledge gap by examining the dispersion of a range of CNTs in the presence of DOM extracted from freshwater.

CNTs ranging in diameter (10-500 nm) and length (1-40 microns) were mixed in solutions of a common surfactant (1% SDS), a commercially available DOM (Suwannee

River DOM), and DOM extracted from Black River. These samples were allowed to settle in the dark for 68 hours. Absorbance spectrometry (UV-VIS) was used to determine the concentrations of CNT in solution over time, after calibration to prepared standards in each medium. The results show that after ~5 hours only 5-30% of all sizes of CNTs were left in 1% SDS solution, while the DOM solutions contained between 46-77%. It was also found that CNTs settled out of the DOM solution faster with increasing diameter. These results demonstrate that CNTs will stay suspended in freshwaters much longer than suggested by studies performed in SDS. The work also shows that the diameter of the CNTs and likely also the structure of the DOM are critical variables affecting dispersion.

Introduction

The production and use of non-functionalized multi-walled carbon nanotubes has reached industrial levels and is expected to increase exponentially into the foreseeable future. For example, as of 2007, Showa Denka of Japan is manufacturing CNT's at a rate of 40 mega tons/year and Raymor industries in Canada produced 3.5 tons in 2006. The market for multi-walled carbon nanotubes is estimated at US $290 million for 2006 and the global demand for CNTs is expected to exceed US $1.9 billion by 2010 (Global Industry Analysts, 2007).

CNTs have many commercial uses including: motor vehicle fuel system components, specialized sports equipment, nanoscale electronics, nylon 12, acetal, fluoropolymers, fluoroelastomers, fuel pumps, O-rings, and paintable exterior automotive body panels. As CNTs become commonplace, the range of uses is expected to expand dramatically (e.g. nanocarriers for targeted drug delivery) as will the potential for release into ecosystems. Currently only a few studies have examined CNT behaviour in the environment and these have primarily been limited to atmospheric transport and toxicity through inhalation or dermal contact (Oberdorster *et al.*, 2007; Maynard *et al.*, 2007, Sharma *et al.*, 2007). However, there is growing concern about the effects of nanomaterials in aquatic ecosystems (Moore, 2006). Jia *et al.* (2006) found that single-walled carbon nanotubes and multi-walled carbon nanotubes can result in cytotoxicity to aveolar macrophage after six hours of *in vitro* exposure. Smith *et al.* (2007) found that single walled carbon nanotubes are a respiratory toxicant in rainbow trout, causing toxic effects after 10 days' exposure at concentrations ranging 0.1 - 0.5 mg L^{-1}. In general, the lack of aquatic toxicity data combined with a lack of research on basic properties of nanomaterials in aquatic ecosystems is the root of our current inability to perform meaningful risk assessments on this class of substances.

Some of the fundamental properties controlling the fate of carbon nanomaterials in freshwaters are just beginning to be explored. For example, a recent study examining CNTs in simulated freshwaters found that CNTs displayed increased dispersion in the presence of dissolved organic matter, thus increasing their ability for transport (Hyung *et al.*, 2007). However, it remains unclear how the physical and chemical structure of CNTs and dissolved organic matter (DOM) will affect the dispersion of CNTs in a natural aquatic environment. These are key issues in order to assess the dispersion of CNTs in

aquatic ecosystems and to provide the fundamental research required for relevant toxicological studies to take place. Several government departments have acknowledged that these are fundamental gaps in research which must be filled in order to assess the risk of nanomaterials to ecosystems (Owen and Handy, 2007).

Our research aims to close this knowledge gap by examining the dispersion of a range of CNTs in the presence of DOM extracted from freshwater. DOM was extracted from Black River near Wolfville, NS. CNTs ranging in diameter (10-500 nm) and length (1-40 microns) were mixed in solutions containing either 1% sodium docecyl sulfate (SDS), a commercial DOM, or Black River DOM and allowed to stand for 68 hours. Samples were filtered and the CNTs that were dispersed in solution after the settling period were quantified. This poster will overview the preliminary results obtained using these methods.

Methods

Three solutions were examined to explore the effect of the matrix on CNT suspension: (i) 1% sodium docecyl sulfate; (ii) commercially available Suwannee River DOM; and (ii) DOM extracted from Black River, Gaspereau, NS. On July 4th, 2007, 500 L of water was sampled from Black River (0389954E; 4987052N) using pre-cleaned HDPE containers. Water chemistry parameters were recorded using a YSI multiprobe system (Temperature = 20 °C; Specific conductivity = 27 µS cm^{-1}; dissolved oxygen 10.1 mg L^{-1}; pH = 6.8; ORP = 147). Portable reverse osmosis (O'Driscoll et al, 2006) was used in the lab to concentrate DOM from the Black River samples. The reverse osmosis concentrate was desalted by placing the sample in dialysis tubing (3500 MWCO) and submersing in excess distilled deionised water for 24 hours.

Carbon nanotubes were purchased with the following outer diameters and lengths: (i) 10-30 nm diameter / 5-20 µm length (lot# 504); (ii) 80-200 nm / length unknown (lot# 1190JN); and (iii) 240-500 nm / length unknown (lot# 1188JN). A 500 mg L^{-1} solution of each CNT was prepared in a glass flask for each matrix. The following dilutions were prepared in 10 mL polypropylene tubes from this stock solution: 250, 50, 5, 0.5, 0.05, 0.005 and 0.0005 mg L^{-1}. All solutions were sonicated for five minutes and stirred to achieve maximum suspension prior to measuring the absorbance between 200-800 nm using an Ultrospec 4300 Pro UV-VIS spectrophotometer. The maximum absorbance for each CNT sample was used for calibration and subsequent sample measurements. All readings were blank corrected using the appropriate matrix.

During the settling experiment, each of the nine solutions (3 CNTs x 3 matrices) was vigorously shaken and then allowed to settle over a 68-hour period. Sub-samples were taken for analysis from each settling solution every hour for the first five hours and every 4-6 hours thereafter. The sub-samples were measured at the wavelength of maximum absorbance based on the absorbance scans for each CNT standard solution (generally 400, 600, or 800 nm). The absorbance scans and calibration curves were normalized to a scale of one to account for small variations in analysis sensitivity. Concentrations in solution were calculated and plotted in MS Excel.

Several samples were prepared on silicon wafers and analysed using scanning electron microscopy (SEM; model JEOL 5900LV) at the Acadia Center for Microstructural Analysis (ACMA) in order to confirm the presence of CNTs suspended in solution and to provide a visual examination of CNT interactions with DOM.

Results and Discussion

Interactions of CNTs with DOM

Figure 1 shows SEM images of samples taken from a solution of 10-30 nm CNTs and Suwannee River DOM which were dried on silicon wafers. The solution was sub-sampled after several days of settling. The SEM image of the dried sample clearly shows CNTs associated with the outside surface of the dried organic matter. This confirms the presence of CNTs in suspension and suggests that they may be associated with the dissolved organic matter (DOM). Other results from the settling experiment also suggest that DOM is key to the increased suspension of CNTs. A comparison of the CNTs in the 1% SDS solution (Figure 2) with the CNTs in both the Suwannee River DOM and the Black River DOM solution (Figures 3 and 4) shows that all sizes of CNTs settle more quickly in the SDS solution than in both DOM solutions. In fact, >70% of CNTs have settled out of solution after five hours in the 1% SDS, as opposed to only 17 - 38 % in the DOM solutions. These results are consistent with the recent work of Hyung *et al.* (2007), who observed that CNTs are readily dispersed in Suwannee River DOM and stay suspended longer there than in 1% SDS.

Effect of CNT Diameter

Initially, in the 1% SDS solution the smallest diameter (10-30 nm) CNTs fall out of solution much more quickly than the 80-200 nm and 240-500 nm CNTs (Figure 2). After ~5 hours >70-95% of all CNTs had settled from the solution with the larger CNTs staying preferentially dispersed. After 68 hours > 95% of all CNTs had settled out of solution (Table 1).

The opposite trend was observed in both of the DOM solutions, in that all CNTs remained dispersed for a substantial period of time and the largest CNTs settled out of solution more quickly than smaller CNTs (Figures 3 & 4). After 5 hours' settling, 46 and 70% of the 240-500 nm CNTs were left in the SR DOM and BR DOM, respectively, while 80-200 nm CNTs had 52 and 77%, and the 10-30 CNTs had 85 and 93% left in solution (Table 1). These results suggest that studies of CNT dispersion performed in SDS alone do not accurately represent freshwaters.

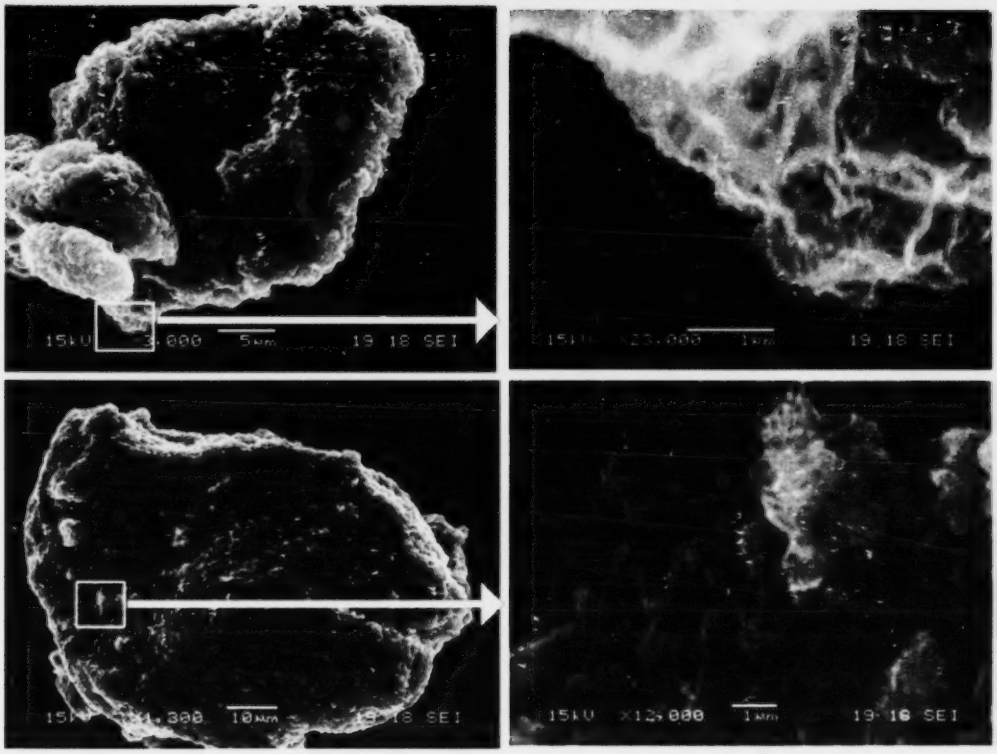

Figure 1: Scanning electron microscope images of carbon nanotubes (10-30 μm diameter) associated with outer surface of Suwannee River dissolved organic matter (after drying on silicon wafer).

toxicity may also be related to size. Templeton *et al.* (2006) found that there was a significant reduction in the life cycle molting and increased mortality of the estuarine copepod *Amiphiascus tenuiremis* when exposed to the fluorescent fraction of single-walled carbon nanotubes. This suggests increased toxicity from the exposure of copepods to smaller size fractions of CNTs. As such, the dispersion of small diameter CNTs in aqueous ecosystems may be particularly important for future risk assessments.

Effect of DOM Structure

The results at five hours show more CNTs dispersed in Black River DOM as compared to Suwannee River DOM; however, this trend disappears as the experiment approaches 68 hours (Table 1). We hypothesize that CNTs are interacting with largely nonpolar areas in the DOM (areas of high aromaticity). We are currently examining the structure of the DOM with the hypothesis that BR DOM has higher molecular weight and is more aromatic in structure than Suwannee River DOM. If this is the case, then it is a plausible explanation for the increased dispersion of CNTs in Black River DOM observed here.

Table 1: Percentage of CNTs remaining in solution after 5 and 68 hours settling time grouped by (a) matrix and (b) diameter of CNT

Matrix	CNT diameter	5 Hours	68 Hours
	nm	% CNT in solution	% CNT in solution
1% SDS	10-30	5	0
	80-200	21	2
	240-500	29	5
SR DOM	10-30	85	75
	80-200	52	29
	240-500	46	46
BR DOM	10-30	93	75
	80-200	77	36
	240-500	70	36

CNT diameter	Matrix	5 Hours	68 Hours
nm		% CNT in solution	% CNT in solution
10-30	1% SDS	5	0
	SR DOM	85	75
	BR DOM	93	75
80-200	1% SDS	21	2
	SR DOM	52	29
	BR DOM	77	36
240-500	1% SDS	29	5
	SR DOM	46	46
	BR DOM	70	36

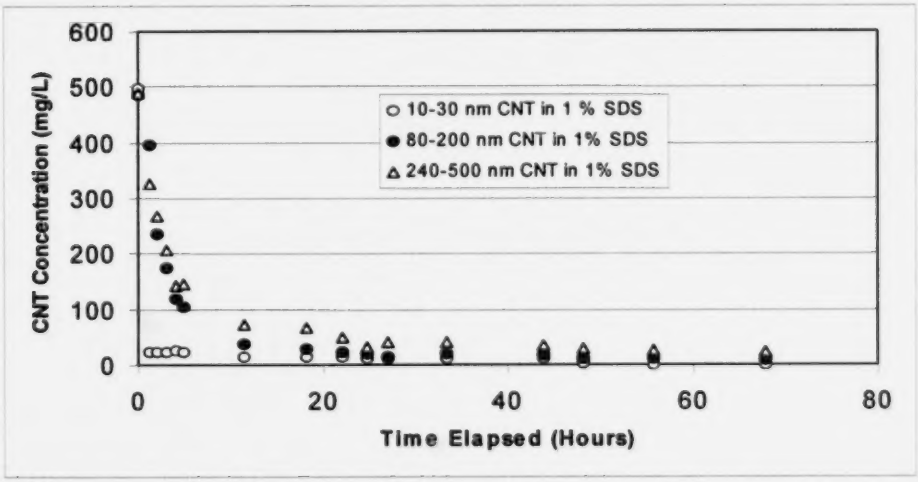

Figure 2: A fast decrease in CNT concentrations suspended in solution was observed during 68 hours of observations in a 1% sodium dodecyl sulfate (SDS) matrix.

The results show that smaller-diameter CNTs are preferentially dispersed in DOM solutions and therefore likely more available for transport and biological interactions in freshwaters. This is particularly important since recent work has suggested that CNT

Significance

This research confirms the work of Hyung et al (2007) which identified that DOM is critical to increased suspension of CNTs. This research is the first to identify that CNT diameter has a significant effect on CNT suspension in freshwaters and that DOM structure may be important to CNT suspension. Our research shows that smaller diameter CNTs will have greater potential for dispersion in freshwaters and ultimately higher potential for exposure and toxic effects. Future work will examine a wider range of CNTs and other nanomaterials for interactions with DOM of varying structures.

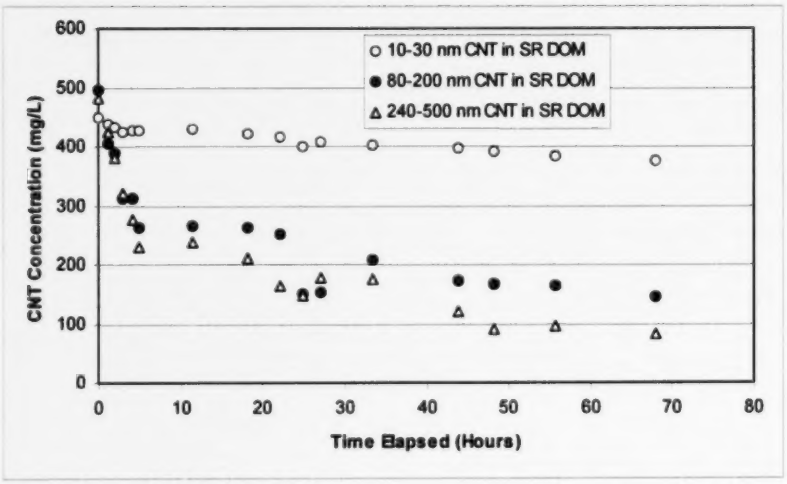

Figure 3: A slow decrease in CNT concentrations suspended in solution was observed during 68 hours of observations in Suwannee River Dissolved Organic Matter (SR DOM) matrix. The smallest diameter CNTs (10-30 μm) were observed to stay suspended substantially longer.

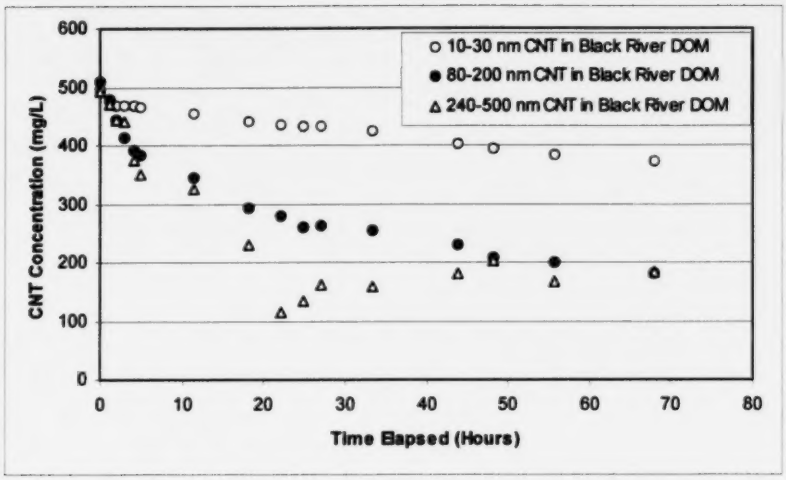

Figure 4: A slow decrease in CNT concentrations suspended in solution was observed during 68 hours of observations in Black River Dissolved Organic Matter (DOM) matrix. The smallest diameter CNTs (10-30 μm) were observed to stay suspended substantially longer.

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Evaluation of metals bioavailability in Botwood Harbour, NL, and potential ecological impacts: an application of the weight of evidence approach based on a marine sediment quality triad. C. Moore[1], L. Marshall[1], R. Willis[1], G. Gilron[2], K. Doe[3], and K. Edwards.[4] (PO)

[1] Intrinsik Environmental Sciences Inc., Halifax, NS; [2]Teck Cominco, Vancouver, BC; [3]Environment Canada, Moncton, NB; [4]Transport Canada, St. John's, NL;

An assessment of the potential impacts of metals on marine sediments and associated ecosystems in Botwood Harbour, NL, was conducted based on comprehensive field- and laboratory-based studies carried out in October, 2004. The studies, which contained key elements of the Sediment Quality Triad (SQT), included: analyses of metals in bulk sediments and sediment porewaters, marine sediment toxicity tests (i.e, *Eohaustorius estuarius* and *Lytechinus pictus* sediment exotoxicity tests), and a bioaccumulation assay using *Macoma baltica*. Sediment samples were also analysed for ammonia, sulfide, redox, AVS/SEM, grain size, and total organic carbon. The results of previously conducted field surveys of the benthic invertebrate community were also considered. Using a weight-of-evidence approach, an evaluation was conducted to interpret the results of these study elements. The study concluded that although bulk sediment concentrations and porewater concentrations for one metal (lead) suggested a potential for adverse biological effects, the toxicity test data clearly indicated that the most contaminated zones of the harbour did not display acute or chronic toxicity to benthic species. It was also concluded that there would be limited bioaccumulation of metals in marine invertebrates, and subsequently in marine fish, and higher trophic level species. Based on the weight of evidence evaluation, it was recommended that sediments be left in place to undergo natural burial. However, monitoring was recommended at periodic intervals to verify if natural sedimentation processes and the burial of contaminated sediments are effectively mitigating potential metals exposure.

Water quality patterns in four areas of northwest British Columbia. M. Whelly[1], R. Goad[2], R. Johnstone[3], D. Stoopnikoff[4] and P. Tredger[5] (PO)

[1]Rescan Environmental Services, Vancouver, BC; [2]Fortune Minerals Ltd., London, ON ; [3]Western Keltic Mines Ltd., Vancouver, BC; [4]NovaGold Canada Inc., Vancouver, BC; [5]Thompson Creek Mining Ltd., Fraser Lake, BC

Water quality is a key indicator of environmental conditions and is used to monitor environments that may be impacted due to anthropogenic stress. Baseline water quality monitoring was conducted on watercourses in four areas of northwest British Columbia for one or more years between 2004 and 2006. In many cases, existing federal and BC provincial water quality guidelines were regularly exceeded for several variables, including sulphate and various metals. This natural phenomenon is related to the high degree of mineralization in the region acting as an erosional source to these watercourses. Exceedance of existing guidelines under pre-development conditions points to the need for developing site-specific water quality objectives for these watersheds, as directed by Environment Canada. This process provides a more accurate indication of natural background ranges above which potential adverse effects could be observed. Water quality datasets were summarized for one watershed from each of the four study areas. Selected variables of interest are plotted to show concentration ranges among the areas, highlighting regional variation. The frequency of exceeded guidelines is provided for the available water quality data set. Seasonal monthly plots are also displayed to show temporal trends, which should also be considered in developing site-specific water quality objectives.

Identification of water quality trends contributing to cumulative effects in the Athabasca River Basin. A. Squires[1], C. Westbrook[1] and M. Dubé[1] (PO)

[1]University of Saskatchewan, Saskatoon, SK

Alberta is currently experiencing significant economic growth and increasing awareness of water dependencies. As a result, novel approaches addressing cumulative effects over broad temporal and spatial scales in these aquatic ecosystems are required. The goal of this research is to develop a quantitative approach to assess and characterize the cumulative effects of man-made stressors on indicators of aquatic health for the Athabasca River Basin in Northern Alberta. There have been increasing levels of industrial (forestry/pulp and paper, coal mining, oil, natural gas and oil sands mining), urban and other land-use related development (agriculture, tourism, wildlife trapping, and hunting) within the Athabasca River Basin. Much of the historical data for the Athabasca River Basin has been integrated but not analyzed using GIS and statistical techniques. The main objective of this work is to quantitatively assess and characterize the trends in water quality over the entire Athabasca River Basin and to compare these trends across two time points (pre and post development). Water quality data was obtained from federal, provincial and various independent groups and was consolidated, standardized and adjusted for seasonal variations in flow rates. The Seasonal Mann Kendall test was applied to assess trends in selected water quality parameters across the river basin over two time points. From this, we can begin to quantify the dominant natural and man-made stressors potentially affecting responses of aquatic biota as well as place the magnitude of any changes into an appropriate context relative to temporal and spatial trends in variability.

Current state of fish contaminant monitoring programs in Canada. E. Jasinska[1] and A. Holzapfel (PO)

[1]Golder Associates Ltd., Edmonton, Alberta

In Canada, 18 ongoing and 16 completed large-scale fish tissue contaminant studies were identified. The one country-wide program is the Environmental Effects Monitoring (EEM) which requires pulp and paper mills and metal mines to conduct monitoring under the Fisheries Act. Dioxins, furans and metals are the targeted contaminants of fish tissues in the EEM program. Currently, there are no monitoring programs to evaluate the country-wide distribution, types, and temporal trends of fish contaminants. On a provincial scale, only Ontario and Quebec have monitoring programs that examine a broad range of fish contaminants (in sportfish). The remaining studies examined either a small number of sites, were short-term, or focused on one or two types of contaminants. Most fish tissue contaminants lack corresponding guidelines linking them with ecosystem health effects. The most significant barriers to examining and interpreting current or historic fish contaminant data in Canada are 1) lack of federal or provincial databases documenting fish monitoring studies, and 2) few studies resulting in published reports. In the USA there are several nation-wide and state-wide fish contaminant monitoring programs which collect comparable data. These data are then stored in publicly accessible internet databases. In Canada, easily accessible federal or provincial databases documenting current and historical fish contaminant monitoring studies would help determine where there is duplication of monitoring effort and which watersheds are being neglected in terms of fish contaminant studies.

The generation and use of exposure scenarios and site-specific data in ecological assessments of chemical substances. D. Boivin[1] (PO)

[1]Environment Canada, Gatineau, QC

Exposure characterization is a major component of the ecological risk assessments conducted by the Existing Substances Division of Environment Canada. Many different exposure estimation approaches can be used to support identification of the risks related to the release of a substance: monitoring data; modeling of site-specific scenarios; modeling using a "unit world"; use of trends or probabilistic approaches; etc. Selection of an approach is often dependent on data availability. This poster looks at development of realistic worst-case-exposure scenarios or distributions-of-exposure scenarios, and examines how different site-specific scenarios and statistical analyses can be used in an ecological risk analysis to support the regulatory decision-making process.

Development of a province-wide framework for source water protection – to protect existing and future drinking water sources in Ontario. M. Nowierski[1], T. Fletcher[1] and A. Weselan[1] (PO)

[1]Ontario Ministry of the Environment, Etobicoke, ON

The Ontario government has passed into legislation Bill 43 (the "Clean Water Act, 2006") to protect drinking water at the source, as part of an overall commitment to protect human health and the environment. Protecting "source water" is the first step in a multi-barrier approach to ensuring the quality, and sustainability, of our drinking-water

supply. A key focus of the government's legislation is the production of locally-developed, science-based assessment reports and source water protection plans. Guidance documents have been drafted that will aid in the development of assessment reports. The assessment reports will identify and categorize the land uses that pose a risk to municipal drinking water sources. The assessment reports will identify the risks that will be addressed in the source water protection plans.

Sediments ecotoxicological risk assessment (ERA) approach for St. Lawrence River. L. Martel[1], M. Babut[2], M. Desrosiers[3], S. Masson[4], C. Bélanger[5] and P. Michon[6] (PO)

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The implementation of the sustainable navigation strategy drawn up under the aegis of the St. Lawrence Action Plan III entails the development of an ERA framework for sediments, taking into account the various management options. Such an approach, using a chemical characterization in the initial stage and bioassays in the subsequent stages, is currently under development. The first management option addressed is the discharge of dredging sediments in unconfined water. In this case, in addition to establishing the level of contamination and toxicity of dredged sediments, it is essential to determine what will become of the deposit and to take into account the hydrodynamic characteristics of the discharge site. Interstitial water and colloids are rapidly dispersed in the water column, whereas particles may form deposits, then be subject to erosion or be covered by the natural sedimentation at the site. As a result, the assessment parameters cover three types of potential impacts: the short-term survival of pelagic organisms, the potential for the recolonization of the deposit by benthic organisms, and the reproductive success of sensitive fish species. This presentation will describe the various stages of the ERA and the measurement tools considered, as well as the future steps in the development of this approach.

Marine snail (Ilyanassa obsoleta) behavioural response to contaminants. S. Marklevitz[1] and J. Hellou[2] (PO)

[1]Dalhousie University, Halifax, NS; [2]Fisheries and Oceans Canada, Dartmouth, NS

Harbours receive multiple sources of contamination where sediments act as a sink for numerous organic and inorganic chemicals. Polycyclic aromatic hydrocarbons (PAH) were previously analysed in Halifax Harbour and levels of many were above the Canadian Council of the Marine Environment sediment quality guidelines. Since a widespread benthic species could not be identified in the field, laboratory exposures were developed to assess the health of inter-tidal invertebrates abundant elsewhere in North America. The horizontal movement of marine snails, described as an

avoidance/preference response relative to contaminated/reference sediments, was investigated. The behavioural response of animals is a sensitive, cumulative biological effect, observed at the organism level. Behavioural changes can result from exposure to neuro-toxic chemicals or contaminants with other modes of action. The sensitivity of the behavioural response can vary with species and type of chemical, but is expected to be more sensitive than an acute narcotic type response. Experiments with mud whelks (*Ilyanassa obsoleta)* yielded distinct and changing avoidance behaviour, with time, towards contaminated sediments. The response was compared to PAH levels in newly analysed sediments and previous source apportionment of these contaminants. Significant avoidance was observed after 24 to 72 hrs of exposure. For lower percentages of harbour/reference mixed sediments, there was a preference for contaminated sediments, while for higher percentages of harbour/reference mixed sediments there was an avoidance of contaminated sediments. The reduction in avoidance is proposed to be in response to the balance between food and contaminants availability in the sediments.

Toxicity testing of sediment collected in the vicinity of effluent discharges from seafood processing plants in the Maritimes. B. Lalonde[1], P. Jackman[1] and K. Doe[1] (PO)

[1]Environment Canada, Moncton, NB

The seafood processing industry is of national economic importance in Canada, with the highest numbers of facilities located in the Atlantic Provinces. The purpose of this study was to collect and analyse both chemical and toxicological characteristics of the sediments to assess potential impacts of seafood processing effluent to the receiving environment. Eighteen sediment samples were collected in the receiving environment near six seafood processing plants. Overall, ammonia levels ranged from <0.2 to 3480 $\mu g \cdot g^{-1}$, sulphide levels ranged from <0.4 to 6970 $\mu g \cdot g^{-1}$, and redox ranged from -255 to 443mV. Microtox™ Solid-Phase Test IC50 ranged from 659 to > 157 000 $mg \cdot L^{-1}$ while amphipod % survival ranged from 0 to 100%. Regression coefficients for the independent variables were negative for ammonia and sulphide, which can be interpreted as an increase in toxicity when ammonia or sulphide levels increased in the sediments. Regression coefficients for redox were positives, which indicate that a decrease in mortality will occur as the redox values increase. The variability explained by the models ranged from 48 to 75%, which indicates that there are other factors which contribute to the toxicity, since a proportion of the variability of the dataset remained unaccounted for.

Agricultural Stressors

Session Co-chairs/ Présidents Alexa Alexander and Glenn Benoy

Methods and development of agri-environmental standards for nutrients and suspended sediment in agricultural streams across Canada. R. B. Brua[1], J. M. Culp[2], P. A. Chambers[1], G. A. Benoy[1] and C. Vis[1] (PL)

[1]Environment Canada, Saskatoon, SK; [2]Environment Canada / University of New Brunswick, Fredericton, NB

Nutrient (nitrogen and phosphorus) and sediment input to aquatic ecosystems can result in deleterious changes in abundance and diversity of aquatic invertebrates and fish. As part of a "National Agri-Environmental Standards Initiative," the Government of Canada has committed to development of non-regulatory environmental performance standards that establish desired environmental quality (for nutrients, sediments, pathogen and ecological flows) for agricultural watersheds. To evaluate methods for setting nutrient standards, an integrated approach has been employed consisting of analysis of existing data from forested and agricultural watersheds in major agricultural ecozones of Canada. For the most part, suspended sediment and total nitrogen and phosphorus increased with an increasing amount of agricultural land cover within the watershed. Similarly, suspended algae (as measured by chlorophyll a content) increased with increasing agricultural land cover as well as increasing nutrient concentrations. Results for various standard-development methodologies and preliminary nutrient and suspended sediment standards for the Maritimes, Ontario and the Okanagan region of British Columbia will be presented. Research is continuing on refining indicators for greater sensitivity and ease of measurement, and developing agri-environmental standards for other ecozones across Canada.

Assessment of the cumulative ecological effects of agricultural stressors on aquatic communities: An elaboration of the Sediment Quality Triad. E. Luiker[1], J. Culp[1] and G. Benoy[1] (PL)

[1]Environment Canada, Fredericton, NB

Field runoff in areas of agricultural activity can carry pesticides, nutrients, and deposited sediments (PND) to adjacent streams. The fate of these stressors on the benthic aquatic environment is various and effects are potentially cumulative. Determining cause-and-effect relationships from multiple agricultural stressors requires an approach that can identify the effects of individual stressors on their own and in combination. To aid in the investigation of cause of these stressors on the stream community, we propose using a weight of evidence approach combining the sediment quality triad to assess pesticides (proposed by Chapman 1990, which includes "chemistry to measure contamination, bioassay to measure toxicity, and in situ biological assessment"), with the novel nutrient

enrichment (water chemistry to measure nutrient concentrations, periphyton Chl a to estimate productivity, and diffusing substrate studies to determine nutrient limitation) and deposited sediment triads (TSS to determine sediment loadings, embeddedness to measure sediment deposition, and benthic community sampling for in situ biological assessment). Effects of pesticide exposure, nutrient enrichment, and depositional sediment accumulation can be estimated using an integrated differential triad response method.

A methodology to interpret watershed-scale pesticides achievable performance standards (APSs) in terms of ideal performance standards (IPSs). A. Rousseau[1], P. Lafrance[1], P. Jiapizian[2], M. Amrani[3], P. Caux[2] and R. Quilbé[1] (PL)

[1]Centre Eau, Terre et Environnement, Quebec, QC; [2]Science and Technology Branch/Direction générale des Sciences et de la technologie, Gatineau, QC; [3]Dir. générale de S&T/S&T Branch, Montreal, QC

The Pesticide Project of the National Agri-Environmental Standards Initiative (NAESI) Program focuses on development of water quality standards at the watershed scale. These standards are APSs and IPSs. IPSs are either based on species sensitivity distribution (SSD) curve or any other ecotoxicological data (e.g., lowest acceptable toxicity test) to identify the desired level of environmental state needed to maintain ecosystem health. APSs represent a set of standards that could be achieved using on-farm Beneficial Management Practices (BMPs). There is a need to link APSs, predicted pesticides concentrations using hydrological modelling, to their potential biological impact in the river.

We recently developed and applied on the Beaurivage Watershed (Quebec, Canada) a hydrological modelling framework to define APSs for pesticides. Using this framework, cumulative distribution frequency curves (CDFC) for Atrazine, Metolachlor and MCPB concentrations were simulated, where CDFCs were obtained after making several simulations (e.g., multiple years) for a period of interest (e.g., summer) and for different stages of BMP implementation. These CDFCs were then used to define APSs, that is, the 90th quartile of a CDFC.

This talk will focus on how to interpret APSs in terms of IPSs—that is, in terms of ecotoxicology and, more precisely, when SSD curves are available. Hence, the discussion will (i) further our understanding of the ecotoxicological meaning of APSs and (ii) provide an approach on how to transfer/communicate these standards to potential end-users (agronomists, farmers, environmentalists, etc.). The idea here is to spell out as clearly as possible for a given APS: How Healthy Will be the Resulting Water?

Validation of surface runoff models using measured pesticide concentrations in agricultural runoff. A. Dunn[1] (PL)

[1]*Environment Canada, Dartmouth, NS*

Since 2001, Environment Canada has been investigating the effectiveness of Prince Edward Island's legislated buffer zones in reducing the toxicity, nutrient, and pesticide load of runoff water from potato fields. Using this data set as a point of comparison, Environment Canada undertook surface runoff model validation for the purpose of identifying a reliable model that can recommend protective buffer zones for fields of varying conditions. Edge of field chlorothalonil concentrations were simulated by surface runoff models, GLEAMS, and PRZM for 19 fields and compared to the maximum measured chlorothalonil concentrations for each corresponding field. Measured chlorothalonil concentrations ranged from 0.1 – 409 $\mu g \cdot L^{-1}$, while simulated concentrations ranged from 2.1 – 30.9 and 2.3 – 457.1 $\mu g \cdot L^{-1}$for PRZM and GLEAMS, respectively. Simulated concentrations were 5 – 37% correlated to measured concentrations. GLEAMS accurately predicted chlorothalonil concentrations with greater frequency, while PRZM generally underestimated chlorothalonil concentrations. A number of data limitations, including inappropriate universal soil loss equation factors, imprecise pesticide application rates and lack of field-specific data (e.g. soil characterization, topography, meteorological conditions) likely compromised the results of this first attempt at model validation. Efforts are being made to gather the sensitive model input parameters retrospectively and in real-time for past and current study sites to allow for more robust model validation work in the future.

CUPS and new pesticides generation: some analytical challenges and environmental fate perspectives. M. Sadiki[1], M. Conruyt[1], J. Bal[1] and L. Poissant[1] (PL)

[1]*Environment Canada, Montreal, QC*

In the last decade, several GC-MS analysis methods for pesticides have been developed with detection limits between 10 and 100 ng L-1 in water (EPA, Method 525.2). These high values of detection limits have allowed for follow-up and evaluation of the contamination of a number of pesticides. In order to improve the method detection limit (MDL) and analyze larger numbers of pesticides, an analytical method using GC-MS has been developed and validated for about 80 compounds such as organochlorines, organophosphorus, carbamates and others.

4L of water samples were filtered and passed through a column containing 10cm of XAD-2. XAD-2 was selected because it allowed retention of a broad range of compounds and also, for high adsorption properties, we can use a large volume of samples. The XAD-2 was soxhlet extracted with dichloromethane for 24 hours. The extract was cleaned up on an alumina column. Samples were analyzed on an Agilent 5975 GC-MS equipped with a PTV injector. The method detection limits (S/N=3) varied between 0.02 and 1.5 ng L-1. The recovery was estimated between 60 and 130% for more than 80% of compounds and the average recovery was about 80%.

This method was successfully applied to samples collected in 2006 in Baie Saint François wetland of the St Lawrence River (Yamaska Basin, Quebec). Pesticides most frequently found and in greater concentration are Atrazine, Metholachlor, Chlorpyrifos, and Demetinamide, and the Atrazine was detected in all samples. This study showed that the GC-MS can give the best detection limits while analyzing a great number of compounds. However, the GC-MS can't be used for analysis of compounds which break up at high temperatures, such as Imazethapyr, Imidacloprid and some new generations of pesticides (sulfonylurea: Rimsulfuron, Nicosulfuron). To analyse these compounds by GC-MS, we have studied their derivatization by BSTFA -TMCS.

Pesticide concentrations in Prairie wetlands and surface water supplies: Results from surveillance studies, 2002-2005. N. Glozier[1], D. Donald[1], A. Cessna[2], E. Sverko[1] and J. Murray[1] (PL)

[1]Environment Canada, Saskatoon, SK; [2]Agriculture & Agri-Food Canada, Saskatoon, SK

Prairie wetlands and drinking water reservoirs located within agricultural land are susceptible to herbicide overspray and atmospheric transport. However, there remains a limited availability of data on the use, sales, occurrence, distribution and fate of current-use pesticides in Canada. Funding was available through the Pesticide Science Fund (2003-2005) to initiate systematic monitoring of priority pesticide residues in the Canadian environment. We report on the results from a study conducted of current-use pesticides (sulfonylurea herbicides, acid herbicides, neutral herbicides and organophosphate insecticides) in small rivers, municipal reservoirs and wetlands in the prairie agro-ecoregion. Two insecticides and 30 herbicides were detected in reservoir and wetland water. The mean concentration of the most frequently detected (82-100%) herbicides in wetlands near agricultural fields were: MCPA 320 $ng \cdot L^{-1}$, 2,4-D 194 $ng \cdot L^{-1}$, clopyralid, 99 $ng \cdot L^{-1}$, dicamba 58 $ng \cdot L^{-1}$, diclorprop 14 $ng \cdot L^{-1}$, mecoprop 9 $ng \cdot L^{-1}$ and bromoxynil 6 $ng \cdot L^{-1}$. Consistent detection of these herbicides suggested that atmospheric deposition, either directly or in rain, was the principal pathway from fields to the reservoirs. These seven herbicides were also the most frequently detected in the reservoirs sampled. For the five herbicides with established drinking water guidelines, all concentrations were well below their respective guideline. However, drinking water guidelines have not been established for the majority of the herbicides found or for mixtures of pesticides. The results from this study have been used as a starting point for the establishment of environmentally relevant standards for herbicide mixtures in prairie wetlands though a wetland mesocosm approach.

Insulate or Exacerbate? Exploring nutrient masking of contaminant effects. A. Alexander[1], J. Culp[2], E. Luiker[2], K. Heard[3], D. Hryn[2] and D. Baird[2] (PL)

[1]University of New Brunswick, Fredericton, NB; [2]National Water Research Institute, Fredericton, NB; [3]Canadian Rivers Institute/University of New Brunswick, Fredericton, NB

The size of adult mayflies is governed by environmental conditions experienced by the aquatic larval. Thus, adult mayflies may be useful indicators of the health and success of streams' insect populations. Our primary objective is to determine whether adult characteristics such as body size and fecundity are useful for evaluating the prevalence and magnitude of sublethal effects under conditions of nutrient-contaminant interaction. Specifically, nutrients may mask the negative effects that contaminants, such as pesticides, have on insect feeding and growth through mechanisms of increased food availability and quality. A primary unknown of this nutrient-contaminant interaction is the crossover point where nutrient enrichment can no longer ameliorate the effects of contaminants. We examine this question through a meta-analysis of mesocosm data from studies that evaluated the effects of agricultural insecticides or pulp mill effluents on stream benthos. The paper will report the results of this evaluation and provide insight into the use of adults stream insects as novel indicators for environmental effects monitoring.

Effects of herbicides on free-living (pelagic) and attached algae and bacteria in Prairie wetlands: a mesocosm approach. M. Waiser[1], N. Glozier[1], D. Donald[1], A. Cessna[2], J. Holm[1] and E. Wallace[1] (PL)

[1]Environment Canada, Saskatoon, SK; [2]Agriculture & Agri-Food Canada, Saskatoon, SK

In late May 2005, a wetland mesocosm experiment was set up next to Pond 79 at the St. Denis National Wildlife Refuge, 45 km east of Saskatoon, SK. Eight holding tanks were positioned adjacent to the pond. Herbicides, including thifensulfuron, clopyralid, fenoxaprop and MCPA (individually and as mixtures) were added to each tank simulating direct overspray of a wetland. One holding tank served as a control (no herbicide addition). Attached algae and bacteria (biofilms) were grown on round microscope coverslips. Replicated samples were taken for bacterial (3H thymidine uptake) and primary (14C labeled bicarbonate) production, bacterial (numbers) and algal (Chlorophyll a) biomass, biofilm bacterial diversity (BIOLOG plates), algal protein to carbohydrate ratios, and water chemistry parameters (major nutrients and ions, oxygen, alkalinity, temperature, conductivity, pH) from each tank on days 1, 2, 3, 7, 14 and 21 post-treatment. Initial results suggest that clopyralid had the most negative effect on biofilm Chl a, while clopyralid, fenoxaprop and thifensulfuron had the most negative effects on primary production. Biofilm bacterial production appeared unaffected. All herbicides and combinations had negative effects food quality (protein to carbohydrate ratios). Pelagic Chl a levels, primary production and bacterial numbers and production in all treated

mesocosms were less than the control over the first three days of the experiment. Thereafter, clopyralid and fenoxaprop appeared to have the greatest negative effect on Chl a, while fenoxaprop (singly and mixed with MCPA or MCPA and thifensulfuron) had the greatest negative effect on bacterial numbers and production and primary production. Taken collectively, these data suggest that some of the most commonly used herbicides across the prairie region have the potential to negatively affect microbial biomass and production in prairie wetlands.

Use of acetylcholinesterase as an indicator of pesticide exposure from in situ studies using the freshwater amphipod Hyalella azteca. A. Bartlett[2], J. Struger[2] and V. P. Palace[1] (PL)

[1]Fisheries and Oceans Canada, Winnipeg, MB; [2]Environment Canada, Burlington, ON

Hyalella azteca is a freshwater amphipod that has been widely used in aquatic toxicology due to its sensitivity to a variety of contaminants, ease of culture in the laboratory, and ubiquitous presence in North American freshwaters. In situ exposures of *H. azteca* were conducted to evaluate the impacts of pesticide use at five sites in the Niagara Region of Southern Ontario: Vineland Creek and four locations on Twenty Mile Creek. Several pesticides are routinely detected in the surface waters at these sites, including clopyralid, dicamba, MCPA, 2,4-D, simazine, atrazine, metribuzin, metolachlor, diazinon, chlorpyrifos, and azinphos methyl. Amphipods were caged for one-week exposures in early May (pre-pesticide application), June, July, and August (peak pesticide usage), and late September (post-pesticide usage) of 2005. Exposures were repeated in 2006 with the addition of three sites on Spencer Creek. Acetylcholinesterase activity of amphipods caged in Vineland Creek during peak pesticide usage was 30-50% lower than controls. A preliminary analysis of pesticide residues in surface waters of Vineland Creek indicates the presence of compounds known to inhibit acetylcholinesterase activity, including chlorpyrifos, azinphos methyl, and diazinon. The relationships between survival, acetylcholinesterase activity, and pesticide levels will be elucidated, and the validity of using in situ exposures with *H. azteca* to evaluate pesticide impacts will be discussed.

The effects of agricultural drain water on the physiological stress response in fish: Interactions and effects of multiple chemical stressors. L. Miller[1], J. Rasmussen[1], V. Palace[2], F. Wang[3] and A. Hontela[1] (PL)

[1]University of Lethbridge, Lethbridge, AB; [2]Fisheries and Oceans Canada, Winnipeg, MB; [3]University of Manitoba, Winnipeg, MB

Agriculture drain water is a source of pesticides and, in some geographical areas, of selenium (Se). The interactions and effects of multiple chemical stressors on physiological status, including the physiological stress response (PSR), of fish are not well understood. In this field study, white suckers and forage fish (emerald shiners, fathead minnows, longnose dace, and juvenile white suckers) were collected from

irrigation return flows in summer and fall. Exposure to pesticides and Se, quantified by the inhibition of acetylcholinesterase (AChE) and muscle Se respectively, were assessed along with PSR parameters. Selenium accumulated in white suckers and plasma AChE activities were lower in the fall than the summer. The PSR of white suckers was primarily influenced by site and season, although Se exposure increased plasma glucose levels and pesticide exposure increased liver glycogen levels. The PSR is a good tool to investigate the effects of multiple stressors as it integrates multiple effects into one system. Forage fish can be used as indicators of pesticide contamination and PSR status. (Funded by MITHE-RN and NSERC.)

An assessment of land-use effects on stream metabolism. ***L. Grace[1], J. Culp[2] and G. Benoy (PO)***
[1]University of New Brunswick, Fredericton, NB; [2]Environment Canada / University of New Brunswick, Fredericton, NB

Stream metabolism, which most often is viewed as the ratio of primary productivity to respiration, has gained interest in recent years as an indicator of overall stream health. Although much research has been directed towards assessing the effect that disturbed riparian zones impose on stream metabolism, less is known about how agricultural practices in stream catchments affect metabolic activity and overall stream integrity. Additionally, seasonal differences in the relationship between catchment disturbance and metabolism have not been extensively explored. Our research assesses whether there is a statistically significant relationship between catchment disturbance and metabolism, and whether this relationship is modified by hydrological seasons. Stream metabolism is measured in five streams using an in situ chamber method whereby dissolved oxygen concentrations are recorded every 15 minutes over a 48 hour period. Catchments of the five study streams span a disturbance range of agricultural coverage from 2% to 77% in the Grand Falls region of northern New Brunswick. During the autumn 2006 we found a statistically significant relationship between catchment disturbance and metabolism, with further sampling planned throughout the 2007 ice-free period. Implications of this research include a better understanding of how anthropogenic disturbances affect stream ecosystems and information for the implementation of environmentally sound agricultural practices.

Lethal and sublethal effects of a ternary agricultural insecticide mixture on aquatic invertebrates. ***H. M. LeBlanc[1], J. Culp[2], D. Baird and A. Alexander[1] (PO)***
[1]University of New Brunswick, Fredericton, NB; [2]Environment Canada / University of New Brunswick, Fredericton, NB

Numerous studies have examined the lethal effects on benthic invertebrates of exposure to a single pesticide. However, lethal exposures to single chemicals do not reflect real-world environmental exposures. For example, aquatic systems in agricultural and urban catchments are often exposed to low concentrations of pesticide mixtures. Consequently, lethality is unlikely to be the best predictor of ecotoxicological responses of an organism to contaminant mixtures. We are investigating the effects of a ternary mixture of insecticides (imidacloprid, dimethoate and chlorpyrifos) on grazing mayflies and the

midge *Chironomus tentans*. This pesticide mixture is commonly used during the production of potatoes in New Brunswick. Effects of the pesticide mixture are measured for both lethal (e.g.LC50) and sublethal exposure regimes (i.e. feeding inhibition, growth and size at emergence) in laboratory and mesocosm experiments. In the lethal and feeding inhibition bioassays with mayflies, the effects of exposure to each of the three study insecticides, as well as exposure to all possible combinations of these chemicals, is examined. Mayfly mortality is expected to increase, while grazing rate is expected to decrease as the number of insecticides in the exposure mixture increases. Additionally, dimethoate is expected to be the least toxic of the three insecticides. This research will help provide a better understanding of the impacts of agricultural pesticide application on aquatic systems, including implications for pesticide regulation, given that the effects of pesticide impact are often underestimated by individual chemical toxicity studies.

Examining the relationship between nutrient loading and fish communities in Prince Edward Island estuaries. A. S. Macdonald[1], K. Teather[1], M. van den Heuvel[1], S. Courtenay[2] and D. Holdway[3] (PO)

[1]University of Prince Edward Island, Charlottetown, PE; [2]Fisheries and Oceans Canada, Fredericton, NB; [3]University of Ontario Institute of Technology, Oshawa, ON

Prince Edward Island has a coastline which includes many estuarine habitats. Almost one-half of the land on the Island has been cleared for agriculture, most of this for potato production. Potato farming removes large amounts of organic material from the soil and therefore requires substantial inputs of fertilizer. There is increasing concern about the impact of nutrient enrichment in Prince Edward Island estuaries as eutrophic conditions become increasingly prevalent. There is no information concerning the effects of such conditions on local populations of fish. The objective of this study is to document the relationship between agriculture, nutrient loading (primarily nitrate levels), and ultimately fish communities. Fifteen estuaries characterized by varying concentrations of nitrates were selected as study sites. Levels of nitrates in the streams feeding those estuaries ranged from <1 to 8 $mg \cdot L^{-1}$. Sampling of inshore fish communities was carried out at four sites on each of the fifteen estuaries in June and will be repeated in August. Fish captured per beach seine haul were identified, counted, weighed and released back to the water. Habitat characterization for each station included: water parameters, vegetation coverage and sediment analysis. Further information on nutrient loading and the extent of eutrophication will be obtained for each estuary.

Assessing the impact of agriculture on mummichog (Fundulus heteroclitus) population health in the estuaries of Prince Edward Island. M. Finley[1], S. Courtenay[2], K. Teather[1] and M. van den Heuvel[1] (PO)

[1]University of Prince Edward Island, Charlottetown, PE; [2]Fisheries and Oceans Canada, Fredericton, NB

Prince Edward Island (PEI) is intensively farmed with approximately 646,000 acres of its 1.4 million total acreage cleared for farm use. These practices have created an overabundance of nitrogen and phosphorus in aquatic environments, resulting in eutrophication and reduced habitat quality for fish. The aim of this study is to examine the impact of agriculture and nutrient loading on the health of an estuarine species, the mummichog *(Fundulus heteroclitus)*. Twenty male and twenty female mature mummichogs were collected from seven estuaries across PEI with differing nutrient loadings in both the months of May and June. Length of fish, body weight, gonad and liver weight were taken for measures of overall condition factor and somatic indices. In vitro steroid production and fecundity will be examined as a measure of reproductive health. Liver and brain tissue were taken for biochemical analyses of liver EROD and brain acetylcholinesterase activity as indicators of pesticide exposure. Population age structure will be determined using otoliths and/or scales. Preliminary results indicate that the condition of fish in the medium-impacted sites is higher than either the high-impacted or reference sites. Samples will continue to be collected monthly over the summer and fall of 2007, along with additional habitat characterizations.

Most receiving-water samples contained several different pesticide residues. Triazines and phenoxy herbicides were the most frequently detected pesticides. Endosulphan was detected in all regions of the country, reflecting its continued use. Several pesticides were detected at levels that exceeded CCME water quality guidelines for the protection of aquatic life, including azinphos-methyl, chlorothalonil, endosulphan, atrazine, chlorpyriphos, and 2, 4-D.

Dose response of herbicide mixtures on aquatic wetland communities using mesocosms. E. Wallace[1], N. Glozier[1], M. Waiser[1], D. Donald[1], V. Tumber[2] and B. Schock[1] (PO)

[1]Environment Canada, Saskatoon, SK; [2]National Water Research Institute, Saskatoon, SK

Prairie wetlands in Saskatchewan are located in and around agricultural land and are susceptible to herbicide overspray. The effects of herbicide mixtures are relatively unknown on arthropod, algae and bacterial communities. It is difficult to assess the effects of herbicides in a field study using wetlands, due to their seasonality, variability and complexity in community structure, as well as the difficulty for replication for statistical analysis. Mesocosms offer a method to study treatment effects under field conditions, they provide more realistic conditions than laboratory tests and allow simultaneous studies on the fate and biological effects of pollutants. Mesocosms allow

for a controlled use of high concentrations of herbicides that could otherwise be very detrimental to an entire wetland in a field experiment. Recently a mesocosm system was used to study the effects of dose response to herbicide mixtures. In June 2007 a mesocosm system was used at a wildlife area in St Denis, Saskatchewan. Eight of the most commonly used herbicides were applied: MCPA, clopyralid, 2, 4 –D, dicamba, bromoxynil, dichloroprop, mecoprop and glyphosate. Concentrations ranged from background levels to 1000 times background levels. The mescosms systems were self-sustaining food web systems with depositional habitats. Each mesocosm system consisted of a large plastic tank 0.61 m in height and 1.82 m in diameter that held five smaller steel tanks, or replicates. Each steel tank was inoculated with sediment, benthic invertebrates, and zooplankton. A pump and manifold system circulated the water from the large tank to the replicates. Endpoint measurements examined in this study were algae and bacterial productivity and biomass and density and community structure of zooplankton and benthic arthropods. Supporting variables were water chemistry, temperature, nutrient, ion and pesticide levels. Emerged insects were collected daily for the duration of the experiment. Total counts and family counts were used to detect effects from herbicide exposure. Benefits to using these mesocosm systems included a more detailed and controlled assessment of herbicides in higher-than-normal concentration levels, with environmental realism for effects that would be difficult to analyze in a full field study.

National Water Quality Surveillance Study for waterborne pathogens and associated indicators in agricultural watersheds in Canada. R. Phillips[1], R. Kent[2], T. Edge[2], I. Khan[2] and D. Klonowska[2] (PO)

[1]National Water Research Institute, Gatineau, QC; [2]Environment Canada, Montreal, QC

The National Agri-Environmental Standards Initiative (NAESI) began in 2004 to develop, field-test and recommend science-based non-regulatory environmental performance standards for Canadian agriculture management practices. NAESI standards themes include biodiversity, pesticides, air and water quality. Under water quality, standards are currently being developed for nutrients, sediments, instream flow needs and waterborne pathogens. At the outset, an analysis was undertaken of current indicators used in determining occurrence, quantity, source, and relative risks of waterborne pathogens to human and non-human receptors. With that information in hand, the national team then embarked on Canada's first national waterborne pathogen surveillance program, focusing on detecting and quantifying a broad suite of relevant agriculturally-derived protozoan, bacterial and viral pathogens along with various water quality indicators: microbial, chemical, and physical. A network of Canadian experimental agricultural watersheds posing high pathogen risk to water quality and water uses were selected as research study areas. These watersheds have areas of Intensive Livestock Operations (ILO's)—specifically, intensive dairy, beef, fowl, and pork operations. Surveillance data from 2005-06 and 2006-07 suggest some challenges in expressing

pathogen potential based on existing standard indicators such as *E. coli*. As ILOs and pathogen sources vary, so, too, do the best predictive indicators or combinations thereof. Final recommendations of relevant standards (indicator suites) will be made in 2008 for further testing and application as national water quality performance benchmarks in agricultural waters. This paper will outline some of the preliminary surveillance results as well as some of the key factors in the development and testing of promising indicators for agricultural waterborne pathogens.

Amphibian Ecotoxicology

Session Chair/ Président Natacha Hogan

Effects of de-icing salts (NaCl) on amphibian community structure in Nova Scotia. S. J. Collins[1] and R. W. Russell[1] (PL)

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The decline of amphibian populations is increasing at an alarming rate (Baillie *et al.*, 2004). A recent global amphibian assessment conducted by the IUCN (The World Conservation Union) revealed 43% of populations are rapidly declining (Baillie *et al.*, 2004). Due to their sensitive physiological constraints, complex life cycles, high site-fidelity and low movement rates, amphibian populations are extremely sensitive to environmental change (Blaustein *et al.*, 1994). Amphibians are central components in the ecosystems they inhabit, playing key roles as both predators and prey (Blaustein *et al.*, 2004). Considered valuable biological indicators of habitat quality, worldwide population declines are concerning as they represent the current state of environmental quality and ecosystem health (Blaustein *et al.*, 2004).

Road salts, primarily NaCl, are used extensively as de-icing agents throughout the northern hemisphere; 14 million tonnes of salt annually are applied to North American roads (Environmental Canada, 2001). Excessive amounts of chloride and sodium ions have been demonstrated to damage ecosystem function and structure by inflicting toxic effects on organisms and degrading habitat quality (Jackson & Jobbagy 2005; Kaushal *et al.* 2005). In August 2000, a five-year federal government study in Canada concluded that road salt should be added to the Canadian Environmental Protection Act Toxic Substance List (Environment Canada, 2001).

Chloride ions are highly soluble, easily dispersed through aquatic systems, and are not subject to rapid loss or degradation (Kaushal *et al.*, 2005). Natural background chloride concentrations in fresh water are generally only a few milligrams per liter; concentrations of 250 $mg \cdot L^{-1}$ are considered the maximum limit for the protection of freshwater biota (Environment Canada, 2001). Road salt application has resulted in chloride concentrations greatly exceeding these levels in freshwater systems of many northern localities (Jackson & Jobbagy, 2005; Kaushal *et al.*, 2005).

The objective of this study was to examine how chloride concentrations in ponds, due to application of road salt, influence amphibian community structure and richness in roadside wetlands of Nova Scotia. Seasonal roadside ponds are important amphibian habitats, as many species have preferences for temporary, vernal pools (Gilhen, 1984; Turtle, 2000). Previous work has shown that amphibians exhibit narrow tolerance levels to salinity; elevated levels result in mortality or sub-lethal effects such as reduced fitness (Christy & Dickman, 2002; Sanzo & Hecnar, 2006; Turtle, 2000). Observations from field surveys and results from acute toxicity tests on five amphibian species were analyzed and are reported to quantify the effect of elevated chloride concentrations on amphibian populations inhabiting roadside wetlands.

Methods

Field Methods:

From April through September of 2006, 26 ponds within 60 m of secondary roads or highways were sampled for the presence of amphibian species. Presence was determined if any life stage was detected at a pond. Sampling methods were consistent for every pond; visual day surveys and additionally, for anuran species, night auditory surveys. Adult amphibians, tadpoles and caudate larvae, and eggs of all aquatic species were sampled by dip net or by hand. Search effort was scaled to pond size such that the majority of pond perimeter was searched by dip net. A five-metre perimeter around the pond was systematically searched by lifting and examining debris in search of terrestrial adults. Triplicate water samples collected during spring, mid-summer, and late summer were analyzed using a Hydrolab®.

Acute Toxicity Tests:

Larvae of five amphibian species were subjected to acute, 96 hour LC_{50} toxicity tests conducted at 10 salt concentrations (500, 1000, 2000, 3000, 4000, 5000, 6000, 7500, 8500, and 10,000 $mg \cdot L^{-1}$ NaCl) and a control composed of Halifax tap water (EPA, 2002). Chloride concentration of dechlorinated Halifax tap water was 8 $mg \cdot L^{-1}$ Cl^-. All salt solutions were made from coarse food-grade salt and dechlorinated Halifax tap water. Species tested were: spotted salamander (*Ambystoma maculatum*), wood frog (*Rana sylvatica*), spring peeper (*Pseudacris crucifer*), green frog (*Rana clamitans*), and American toad (*Bufo americanus*). Each treatment for anuran larvae contained 6 individuals with 4 replicates per treatment. Due to the scarcity of similar-sized spotted salamander larvae in local ponds and the cannibalistic behavior of this species, only 4 larvae per replicate were used. Experiments were checked daily, dead individuals were removed, and body weight was recorded. Treatments were observed for physical or behavioral abnormalities. Testing was terminated after 96 hours and all specimens were removed and weighed.

Statistical Methods:

Normality was assessed using the D'Agostino-Pearson method (Zar, 1999). Chloride concentration and road distance data were logarithm transformed to achieve normality. Chloride concentrations in 26 roadside ponds among the three water sampling periods

were analyzed by repeated measures analysis of variance (ANOVA). A stepwise regression was performed where amphibian species richness in ponds was regressed on chloride concentration, distance to any road, distance to major highway, and pH. Individual Mann-Whitney tests were performed for each amphibian species to determine differences in chloride concentrations in occupied vs. unoccupied ponds. Results from toxicity testing were analyzed by the trimmed Spearman-Karber method due to their non-parametric distribution (EPA, 2002). LC_{50} values and 95% confidence intervals for each species were estimated by this method.

Results

Field Surveys:

Mean chloride concentrations in ponds were greatest in the spring, least in mid-summer, and showed a rising trend in late summer (Figure 1). Repeated measures ANOVA showed significant differences in chloride concentrations among ponds ($F_{27, 56} = 392.0$, $p < 0.0001$) and among collection dates for individual ponds ($F_{2, 112} = 118.0$, $p < 0.0001$). There was a significant interaction between pond and collection date ($F_{54, 112} = 12.9$, $p < 0.0001$). Interaction indicated that chloride concentrations did not respond similarly in individual ponds over the study period. Stepwise regression indicated that only chloride concentrations in water had a significant effect on amphibian species richness ($F = 5.52$, $p = 0.027$, $r^2 = 0.19$) (Figure 2). Mann-Whitney tests indicated that there were significant differences in chloride concentration among occupied versus unoccupied ponds for spotted salamanders and wood frogs (both $U = 149.0$, $p < 0.0001$) (Figure 3).

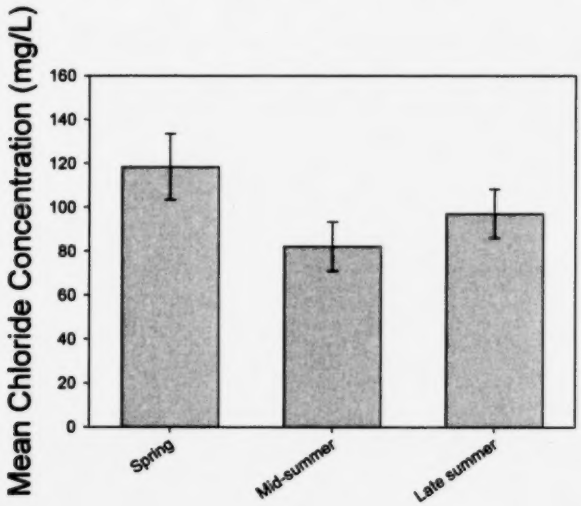

Figure 1: Mean chloride concentrations ($mg \cdot L^{-1}$) over three collection periods in the study ponds. Error bars denote ± 1 standard error.

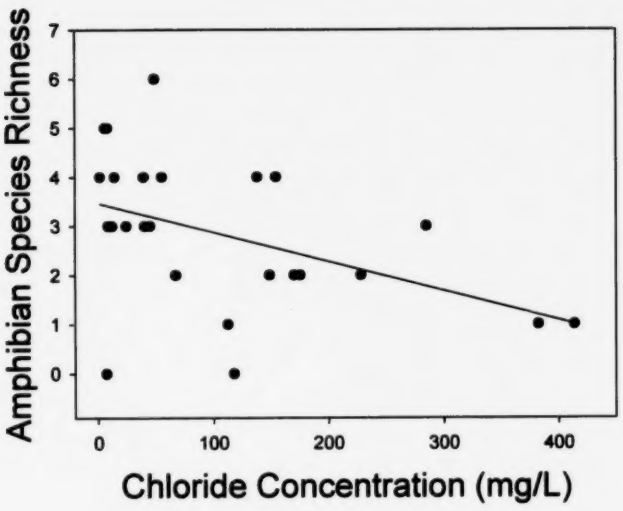

Figure 2: Effects of chloride on amphibian species richness. Regression line is shown.

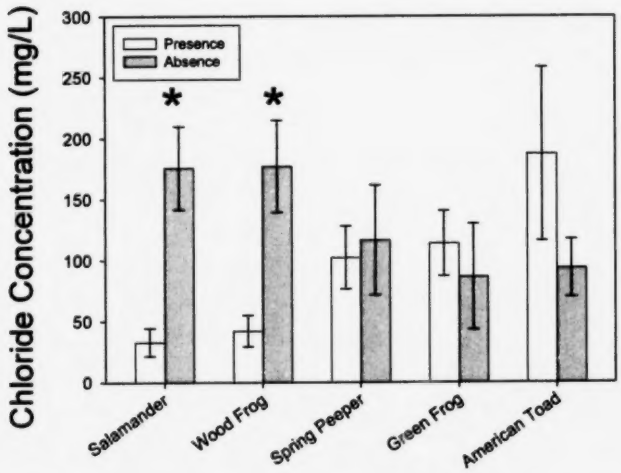

Figure 3: Differences in chloride concentration in occupied and unoccupied ponds for 5 amphibian species.

Acute Toxicity Tests:

The trimmed Spearman-Karber method was used to determine median lethal concentrations (96 hour LC_{50}) (Figure 4). No mortalities occurred in any of the controls for all five species. Spotted salamander treatments produced the lowest median lethal concentration of 1942.15 $mg \cdot L^{-1}$ (95% CI: 1495.77- 2521.79). The next lowest was found for wood frogs at 2837.69 $mg \cdot L^{-1}$ (95% CI: 2437.44- 3303.66). Derived Median lethal concentration for spring peepers was 4665.78 $mg \cdot L^{-1}$ (95% CI: 4091.99- 5320.03) and 5125.49 $mg \cdot L^{-1}$ (95% CI: 4656.12- 5642.18) was found for green frogs. American toads demonstrated the greatest tolerance to salt, producing a median lethal concentration of 6471.50 $mg \cdot L^{-1}$ (95% CI: 6147.42- 6812.66).

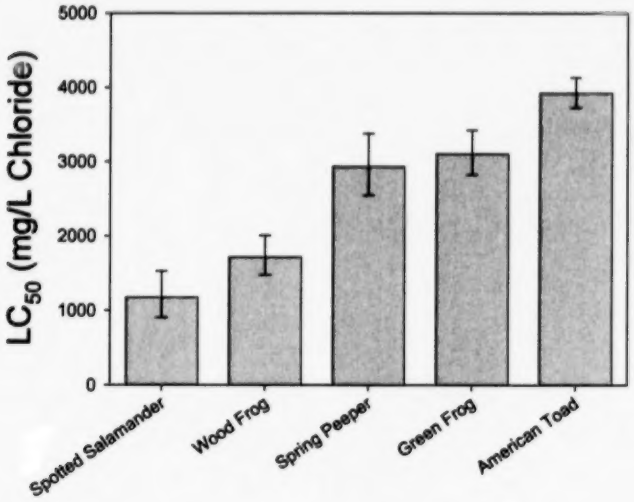

Figure 4: Comparison of median lethal concentration values.

Discussion

Analysis of chloride concentrations within the study ponds indicates that concentrations differ both spatially (between individual ponds) and temporally (between sampling periods). The greatest mean chloride concentrations were measured during spring; this is explained by high inputs of NaCl entering aquatic systems from road runoff and snow melt during this time. Environment Canada (2001) measured chloride levels of over 18,000 $mg \cdot L^{-1}$ in road runoff. High chloride containing runoff during spring may threaten sensitive embryonic and larval stages of amphibians, especially for earlier breeders such as spotted salamanders and wood frogs. Chloride is persistent in aquatic environments and not subject to rapid loss or biological use (Kaushal *et al.*, 2005). Ponds still contained elevated levels in mid-summer, but at lower concentrations than in spring through dilution (by precipitation).

Samples collected in late summer reveal a rising trend in mean chloride concentrations. Pond sizes during this period were decreasing due to evaporation, enhancing chloride levels. Environment Canada (2001) also suggests that by late summer, chloride ions could be reaching surface waters through contaminated groundwater systems. Significant interaction was observed between individual ponds and sample collection dates, indicating that individual pond types respond differently to chloride inputs. Permanent water bodies had more consistent concentrations in both mid- and late-summer collections. Temporary ponds that dry considerably by the end of summer had highly elevated chloride concentrations in late summer. Pond desiccation and consequently elevated chloride concentrations in late summer coincide with the onset of metamorphosis in many species inhabiting these temporary wetlands (Gilhen, 1984; Turtle, 2000). Local populations in temporary ponds may be under time restrictions to reach metamorphosis before the natal pond completely evaporates. It has been demonstrated that exposure to salt may inhibit growth or prolong time to reach metamorphosis (Christy & Dickman, 2002; Gomez-Mestre *et al.*, 2004; Sanzo & Hecnar, 2006).

Chloride concentration was the only factor investigated that was significant in determining amphibian species richness of ponds. Species richness decreased with increasing chloride concentrations. Spotted salamanders and wood frogs were absent from ponds with elevated chloride concentrations, corresponding with toxicity tests identifying these two species as having the lowest LC_{50} values for NaCl.

Comparisons between derived median lethal concentrations showed that the five species tested exhibit different tolerances to chloride; indicating this sensitivity may be a strong factor in determining community structure in wetlands receiving de-icing salt. Spotted salamanders and wood frogs were the most sensitive species. These findings are consistent with other research regarding chloride sensitivities of these species (Sanzo & Hecnar, 2006; Turtle, 2000). Spotted salamanders and wood frogs are more terrestrial than other amphibians (Gilhen, 1984). Spending the majority of their adult phases in terrestrial environments may cause them to have lower tolerances to aquatic contaminants. Species that spend greater portions of their adult lives in the aquatic environment (including hibernation), such as green frogs, have had longer evolutionary time to adapt higher tolerances to fluctuations in water chemistry (such as salt concentration and pH).

Toads displayed high tolerance to sodium chloride (median lethal concentration of 6471.5 $mg \cdot L^{-1}$), and their abundance in roadside pools indicated they are unaffected by chloride concentration. High tolerance could be explained by adaptation to occupying coastal habitats that receive inputs of salt from marine water. It could also be due to evolutionary adaptation of toad oviposition in highly ephemeral ponds (Gilhen, 1984). Other toad species have also been reported to have relatively high salt tolerances (Dougherty & Smith, 2006; Gomez-Mestre *et al.*, 2004).

Behavioral abnormalities were observed in varying degrees between all species tested, in all concentrations. These included delayed responses to stimuli and an overall reduction in normal activity levels such as feeding and swimming, as well as erratic circular swimming and abnormal floating on back or sides. These effects have potential to seriously reduce individual fitness in the wild by increasing risks of predation and disrupting normal activities. Sanzo & Hecnar (2006) reported similar responses in wood frog larvae exposed to NaCl. Previous work has shown that chloride levels not sufficient to cause direct mortality to individuals may still decrease population viability through sub-lethal effects (Christy & Dickman 2002; Gomez-Mestre *et al.* 2004; Sanzo & Hecner 2006). Reductions in activity causing decreased foraging and feeding behaviours could result in decreased growth and development as well as possible failure or prolonged time to reach metamorphosis. Increasing the length of time spent during the larval period decreases an individual's chance of survival, since larval stages are more susceptible to predation and toxicity from aquatic pollution (Brodie & Formanowicz 1983). Individuals of smaller sizes have been shown to be more susceptible to predation (Brodie & Formanowicz 1983). Some of the behaviours observed in the higher concentration treatments may also have the potential to attract predation, such as floating on back and sides, and the erratic, circular swimming observed. Exposure to sub-lethal chloride concentrations therefore may result in the inability of larval amphibians to complete their lifecycles, resulting in the eventual loss of the population.

Results indicate that road salts negatively affect amphibian community structure and species richness by excluding sensitive species from high chloride environments and by inflicting fitness reducing, sub-lethal effects on others. Road salt application is, therefore, a potentially important factor contributing to amphibian declines in northern latitudes.

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Fertilizers and enrichment of nitrogen isotopic signature in larval green frogs. D. Jefferson[1] and R.W. Russell[1] (PL)

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Stable isotopes analysis was performed on green frogs (*Rana clamitans*) at various stages of development, from early larval feeding stages to adults. Changes in C stable isotopic ratios illustrated ontogenetic changes in green frog diets. Enriched N signatures indicated increases in trophic position in response to changes in diet. The isotopic signatures from relatively pristine wetlands differed dramatically from wetland sites receiving nitrogenous pollution. Nitrogen isotopic signatures of green frog tadpoles were greatly enriched relative to tadpoles from unpolluted wetlands, and N signatures were found to be similar to or greater than those of adult green frogs. Presumably, enrichment occurred as a result of prolonged exposure to and consumption of enriched nitrogen from fertilizers by tadpoles. Adult green frogs are not as closely associated with the aquatic environment as tadpoles, and the majority of the adult diet consists of terrestrial invertebrates; therefore exposure to enriched nitrogen from food consumption is limited. Comparison of N stable isotope levels between larval and adult green frogs provides a means to quantify enrichment due to nitrogenous pollution in individual ponds. Nitrate tests conducted on water samples from each pond indicated that these ponds were polluted with excessive nitrogen. Isotopic analysis of sediment samples from each pond indicated similar N enrichment in patterns.

Introduction

Anthropogenic pollution is a serious threat to amphibian communities (de Wijer *et al.*, 2003; Hamer *et al.*, 2004). Nitrogenous pollution has been associated with lethal and sub-lethal effects on many amphibian species (de Wijer *et al.*, 2003; Hamer *et al.*, 2004). A common source of nitrogenous pollution is fertilizer, which is commonly applied to recreational and agricultural lands (Karr *et al.*, 2001). Applied nitrogen could be transported through runoff or leech into groundwaters through the soil (Karr *et al.*, 2001). Green frogs are a biphasic species, meaning their life history is divided into an aquatic larval phase and a semi-terrestrial adult phase (Gilhen, 1984; Jenssen, 1967). Larval green frogs are primarily detritivores and facultative filter feeders that occupy relatively low trophic levels (Jenssen, 1967). Adult green frogs are gape limited predators of terrestrial invertebrates and other amphibians and should occupy superior trophic levels relative to tadpoles (Gilhen, 1984). Green frogs have shown an extraordinary ability to survive in ponds contaminated with nitrate, while similar species are negatively affected (Smith *et al.*, 2006). Analysis of green frog tissues may provide an indication of wetland disturbance.

Stable isotopes are naturally occurring, stable variations of elements that differ only in nuclear mass (Rubenstein & Hobson, 2004). Abundance of stable isotopes is influenced by biological and biogeochemical processes (i.e., fractionation) (Rubenstein & Hobson, 2004). Stable isotopes are integrators of information over time, rather than snapshots of feeding, and can therefore be used to indicate trends in trophic positioning (Kiriluk *et al.*, 1999). δ15N values tend to become enriched between consumer and diet providing an indication of relative trophic levels between species (Kiriluk *et al.*, 1999). δ13C values of a consumer tend to closely resemble that of their general diet (Kiriluk *et al.*, 1999).

It is expected that the isotopic signature of larval green frogs from polluted wetlands will exhibit significantly enriched δ15N values relative to relatively unpolluted wetlands. Additionally, δ15N values of tadpoles from polluted wetlands should resemble those of adult frogs, due to the relatively low N exposure of semi-terrestrial adults (Gilhen, 1984).

Methods

Samples were collected between June and August 2005 from six wetland sites across Nova Scotia; Antigonish (Antigonish county: 45° 34.962' N, 62° 1.238' W), Bass River (Colchester county: 45° 13.268' N, 62° 35.297' W), Bear Cove (Halifax county: 44° 32.585' N, 63° 33.065' W), Berwick golf course (Annapolis county: 45° 4.136' N, 64° 48.396' W), Canning (Kings county: 45° 8.960' N, 64° 26.329' W), and Liscomb game sanctuary (Halifax county: 44° 52.000' N, 62° 34.720' W) (Fig. 1). Collections sites were primarily located through use of maps and global positioning satellite (GPS) coordinates from previous visitation.

Larval, juvenile and adult green frogs were collected from each site. The earliest larval stages obtained were stage 24-25 tadpoles (Gosner, 1960). Specimens were obtained through active pursuit, and using a D-frame aquatic net for manual capture. Three 100-

200 mL water and sediment samples were acquired from each of the wetland sites. In total, 186 samples were collected for this study, 150 tadpoles/frogs, 18 water samples, and 18 sediment samples.

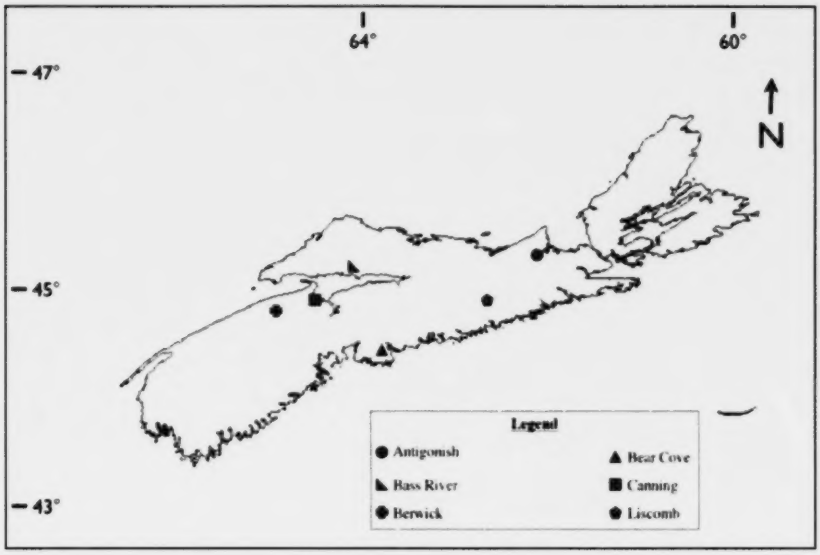

Figure 1: Map of wetland sample locations across Nova Scotia

Specimens were euthanized using a tricaine methane sulfonate (TMS) solution (> 250 $mg \cdot L^{-1}$) buffered to a pH of approximately 7. Adult green frogs were submerged in the buffered solution until euthanized, rinsed and then rapidly frozen in a –80˚C freezer. Frozen adults were coarsely ground using a large tissue homogenizer and the resulting samples were placed into labeled glass sample jars. Small samples (< 1.5 mL) of the homogenized specimens were removed and placed into similarly labeled 1.5 mL micro-centrifuge tubes and stored at –20˚C.

Tadpoles were examined under a dissecting light microscope and staged according to Gosner (1960). Specimens were then placed into labeled, sterile micro-centrifuge tubes or thoroughly cleaned glass sample jars, and tadpole/label information was recorded in a lab book, and in an electronic spreadsheet for later reference. Specimens were frozen and stored at –20˚C. Tadpoles were coarsely ground using a mortar and pestle, then placed into coded micro-centrifuge tubes. Micro-centrifuge tubes, containing the ground samples, were dried at 70˚C for 48-72 hours. Dried samples were ground into a fine powder using a specialized pestle designed to fit within the micro-centrifuge tubes. Whole body samples were used for both tadpole and adult specimens.

Water samples were refrigerated in the lab, and left to settle for 24 hours. Water samples from each of the six collection sites were tested for nitrate (NO_3) concentration. Hach® brand Permachem reagents and a Hach® portable spectrophotometer (DR/2400) were used to test the levels of nitrate.

Sediment samples were left to settle in a refrigerator for 24 hours, after which excess water was decanted from each sample. Small samples (< 1.5mL) of sediment were placed into labeled 1.5 mL micro-centrifuge tubes and dried in a drying oven at 70°C for 48-72 hours. Dried sediment samples were ground into a fine powder using the specialized micro-centrifuge tube pestle.

Finely ground samples were sent to the Stable Isotopes in Nature (SIN) laboratory at the University of New Brunswick, Fredericton, New Brunswick, for analysis. Samples were analyzed using a Finnigan Mat Delta Plus isotope-ratio mass spectrometer (Thermo Finnigan, Breman, Germany) (Jardine *et al.*, 2004).

Values of the stable isotopes ($\delta^{13}C$ or $\delta^{15}N$) are found as the deviance (δ: delta) of the ratio of heavy to light isotopes (i.e., $^{13}C/^{12}C$ or $^{15}N/^{14}N$) within a sample, from that of a standard, and expressed in parts per thousand or per mil (‰) (Jardine *et al.*, 2004). Stable isotope values are calculated using the following formula:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

Where X is ^{13}C or ^{15}N, and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Jardine *et al.*, 2004). International standards for the calculation of stable carbon and stable nitrogen isotopes are Vienna Pee-Dee belemnite (VPDB, 1.95‰) and atmospheric nitrogen (N_2, 0‰), respectively (Jardine *et al.*, 2004).

Tadpoles of all developmental stages, for each location, were grouped for statistical analysis. Kruskal-Wallis tests were performed to determine if there were differences in $\delta^{13}C$ and $\delta^{15}N$ values between tadpoles, newly emerged, and adult green frogs from Berwick, Canning, and Liscomb locations. Mann-Whitney tests were used for similar analyses of values between life stages, from Antigonish and Bass River (where no newly emerged frogs were found), and Bear Cove (where only tadpoles and newly emerged life stages were available). A conservative testing procedure was adopted where the experiment-wise error was Bonferroni corrected to $\alpha = 0.008$, to reduce the risk of committing a type I error.

Results

Analysis of wetland water chemistries indicated sites in Berwick and Canning had greater nitrate concentrations relative to the other four wetlands (Fig. 2). Canning exhibited a vastly higher nitrate concentration than all other wetlands, including Berwick (Fig. 2). Tadpoles and sediment samples from Berwick and Canning exhibited nitrogen isotopic signatures that were relatively enriched compared to those of the other wetlands (Fig. 3 and Fig. 4). In Berwick, tadpoles exhibited $\delta^{15}N$ values that were not significantly different from those of adult green frogs ($H = 8.96$, $df = 2$, $P = 0.01$), while in Canning tadpoles exhibited $\delta^{15}N$ values that were significantly enriched relative to adult green frogs ($H = 12.88$, $df = 2$, $P = 0.002$).

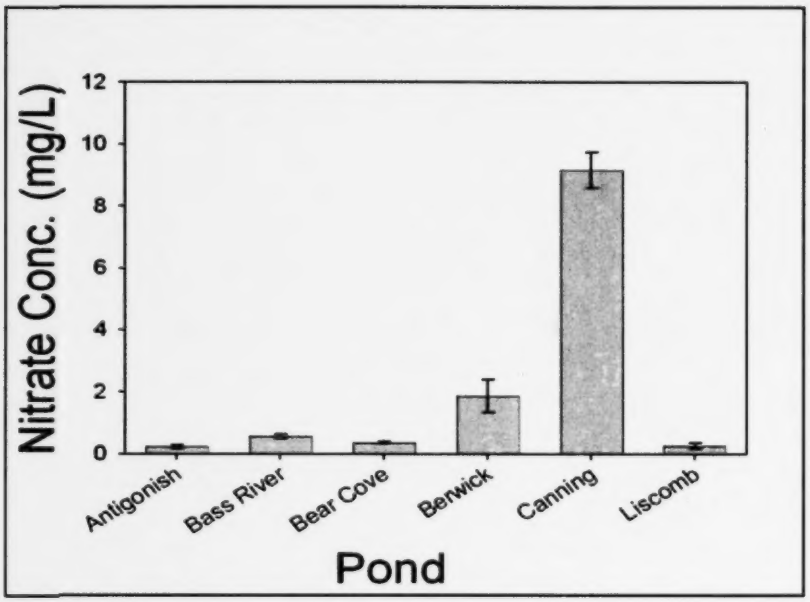

Figure 2: Nitrate concentrations observed from water samples of each of the wetland sampling sites. Error bars represent ± 1 S.E.

Discussion

Lands surrounding ponds in Berwick, and Canning are associated with fertilizer application. Berwick is a water trap on a golf course, while agricultural lands surround the pond in Canning. Nitrate concentrations of these wetlands strongly suggest nitrogen pollution from fertilizer application to the surrounding lands. This supports the hypothesis that $\delta^{15}N$ values of larval green frogs were enriched from exposure to anthropogenic nitrogen pollution.

Given the proximity to recreational and agricultural lands, there is a high probability that this pollution originated from fertilizers, and the nitrogen was carried into the wetlands in runoff waters. These results also appear to indicate differences in the types of fertilizers used at each site (Karr *et al.*, 2001). Organic fertilizer (manure) exhibits relatively enriched $\delta^{15}N$ values compared to inorganic fertilizers because this nitrogen has been exposed to numerous biological processes resulting in enrichment (Karr *et al.*, 2001). Inorganic fertilizers are produced with atmospheric nitrogen, which typically has a $\delta^{15}N$

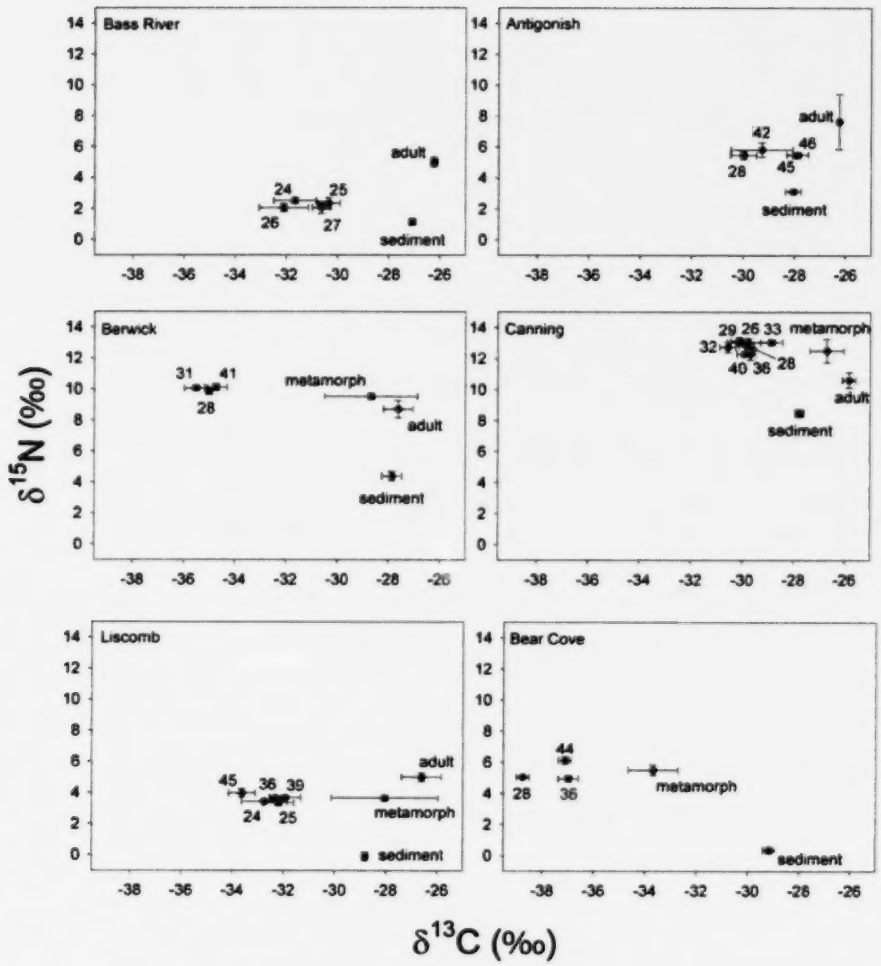

Figure 3: Comparison of $\delta^{13}C$ and $\delta^{15}N$ values for green frogs and sediment across all study wetlands. Gosner stage numbers and descriptions beside each data point indicate green frog developmental stages. Circles indicate the mean value and standard error bars indicate ± 1 S.E.

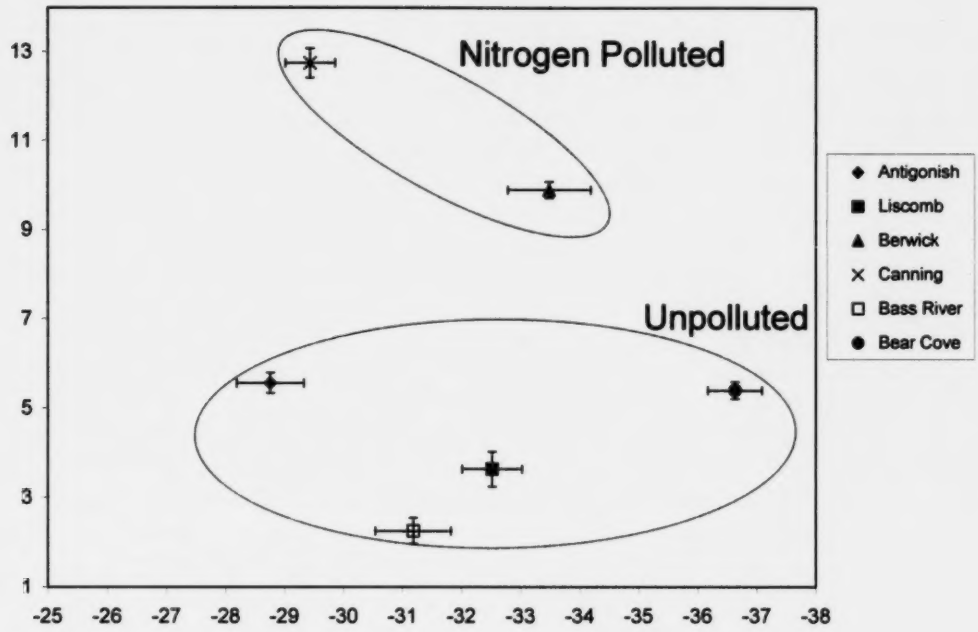

Figure 4: Comparison of pooled tadpole $\delta^{13}C$ and $\delta^{15}N$ values (within each wetland) between nitrogen polluted and relatively unpolluted wetlands. Symbols indicate the mean value and standard error bars indicate ± 1 S.E.

value of approximately 0‰ (Karr *et al.*, 2001). Biochemical processes within the soils could enrich nitrogen from an organic fertilizer source to approximately 10-20‰, while nitrogen from inorganic fertilizer typically exhibits signatures between 4-8‰ (Karr *et al.*, 2001). Lower enrichment observed in Berwick appears to correspond to that which would be expected from exposure to inorganic fertilizers, while Canning exhibits far greater enrichment, which would be associated with the organic fertilizers. It is to be expected that organic fertilizers would be more closely associated with agricultural lands, and inorganic fertilizers with a golf course.

Literal interpretation of tadpole nitrogen signatures from Berwick and Canning would imply that these tadpoles occupy equivalent or superior trophic levels, respectively, compared to adult green frogs. Given that tadpoles are primarily detritivores and adults are carnivores, it is unlikely tadpoles could be considered trophically superior to adult green frogs. However, without reference to relatively unpolluted wetlands the true trophic positioning of these animals would be unclear.

Nitrogenous pollution has an enrichment effect upon nitrogen isotope signatures of exposed individuals. Interpretation of stable isotopic signatures must therefore be carefully considered when interpreting experimental results. Site location and condition

should be carefully considered, as well as the particular biological and ecological aspects of study specimens.

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Regulation of aromatase and estrogen receptors in the brain of developing <u>Xenopus tropicalis</u>: implications for endocrine disruption. P. Duarte[1], N. Hogan[2], B. Pauli[3] and V. Trudeau[1] (PL)

[1]University of Ottawa, Ottawa, ON; [2]University of Prince Edward Island, Charlottetown, PE; [3]Environment Canada, Ottawa, ON

There is now a consensus that amphibian populations have declined since the 1960s. It has been proposed that endocrine disrupting chemicals (EDCs) are affecting tadpole development and are contributing to these declines. The enzyme aromatase (Arom) and estrogen receptors (ERs) are important components of vertebrate sexual development and are potential targets for these EDCs. Using real-time RT-PCR, we compared brain Arom, ERα and ERβ mRNA expression over five different stages of *X. tropicalis* development

(from premetamorphosis to juvenile). All transcripts were detected in the brain throughout development; levels were significantly higher at metamorphic climax and in juveniles compared to tadpole stages. Because there is evidence that disruption of the thyroid system affects sexual differentiation in *anurans*, we also measured Arom and ERs expression in prometamorphic tadpoles following exposure to thyroid hormone (T3 at 0.5, 5, or 50 nM for 48 hours). Exposure to T3 resulted in a dose-dependent increase in ERα and ERβ expression; however, there was no change in Arom expression. These results suggest that T3 contributes to the regulation of ER expression in the brain of developing *X. tropicalis*. Results from a parallel experiment in *Rana pipiens* support this concept. However, in that experiment Arom was down-regulated, suggesting that T3 regulation of Arom is species-specific. This work suggests that cross-regulation between thyroid and estrogen pathways is important for normal amphibian development and should be considered when examining endocrine disruption in amphibian species. Supported by NSERC, Canadian Water Network, and U-Ottawa.

Gonadal development in Rana pipiens tadpoles and the influence of Aatrex® (Atrazine) and Roundup® (Glyphosate) exposure. ***C. Fridgen[1], M. Berrill[1], K. Doe[2], P. Jackman[2] and B. D. Pauli[3] (PL)***

[1]Trent University, Peterborough,ON; [2]Environment Canada, Moncton, NB; [3]Environment Canada, Ottawa, ON

There is increasing interest in the use of amphibians in pesticide and other contaminant monitoring and regulatory programs assessing potential effects on the reproductive and thyroid axes. While stunted growth, delayed metamorphosis, and gonadal abnormalities have been reported following amphibian exposures to pesticides, the lack of background information concerning "normal" values needs to be addressed. In this study we are investigating the growth and development of Northern Leopard Frogs *(Rana pipiens)* reared in the laboratory with or without exposure to pesticides. Results show that even under controlled laboratory rearing conditions, significant variability in growth parameters of unexposed animals can be observed; however, significant effects from exposure to common pesticides can still be detected. For instance, exposure to atrazine (Aatrex® end-use formulation) significantly increased ovary size and section surface area, while testis size decreased in surface area. Abnormal gonad prevalence was also significantly increased in tadpoles exposed to environmentally relevant concentrations of Atrazine (0.1 AI $\mu g \cdot L^{-1}$ and 1.8 AI $\mu g \cdot L^{-1}$) and Roundup® (0.6 AI $mg \cdot L^{-1}$ and 1.8 AI $mg \cdot L^{-1}$). Both contaminants also exhibited significantly different effects on morphometrics between the sexes, reiterating the importance of histology in detecting endocrine disrupting contaminants. By comparing the contaminant effects to "natural" development and differentiation in *Rana pipiens*, our results confirm that low levels of Aatrex® and Roundup® formulations influence amphibian development.

The development of a standardized acute toxicity test with the leopard frog (Rana pipiens) and comparison of the results with standard test species. P. Jackman[1], K. Doe[1], B. Pauli[2] and R. Scroggins[1] (PL)

[1]Environment Canada, Moncton, NB; [2]Environment Canada, Ottawa, ON

Certain amphibian populations are declining. Adverse effects resulting from exposure to pesticides have been cited as one possible reason for these declines. It is difficult to establish guidelines or "safe levels" for the protection of native amphibians from contaminants, as their general sensitivity to these compounds is incompletely known. One reason for this is a lack of standardization of the methods used to conduct toxicity tests with amphibians. The Environment Canada laboratory in Moncton, NB, is establishing standard methods for rearing, breeding and testing *Rana pipiens*. Test method conditions are being established for conducting acute toxicity tests (4 days) with *Rana pipiens* tadpoles. To date, twenty-three pesticide formulations and seven other chemicals of concern have been evaluated. Results from these tests were used to compare the sensitivity to pesticides of native amphibians against standard test species (fish, invertebrates, plants, bacteria) and assess whether pesticide regulatory programs based on acute toxicity tests with standard test species are protective of native amphibian species. Preliminary analyses suggest acute toxicity of pesticides to *Rana pipiens* is similar to that of standard test species. For a few chemicals, acute testing has been conducted at various developmental stages to evaluate relative sensitivity of the tadpole during development.

Identifying sensitive periods for estrogenic endocrine disruption of metamorphosis and sex differentiation in the Northern leopard frog (Rana pipiens). N. Hogan[1], P. Duarte[2], M. Wade[3] and V. Trudeau[2] (PL)

[1]University of Prince Edward Island, Charlottetown, PE; [2]University of Ottawa, Ottawa, ON; [3]Health Canada, Ottawa, ON

During the transformation process from larval tadpole to juvenile frog, there are critical periods of metamorphic development and sex differentiation that may be particularly sensitive to endocrine disruption. The aim of this study was to identify sensitive developmental periods for estrogenic endocrine disruption in the Northern leopard frog (*Rana pipiens*) using short, targeted exposures to the synthetic estrogen ethinylestradiol (EE2). Post-hatch tadpoles (Gosner stage 27) were exposed to 5 nM EE2 over five distinct periods of metamorphosis: early (stage 27-30), mid (stage 30-36), early and mid (stage 27-36), late (stage 36-42), and the entire metamorphic period (chronic; stage 27-42). For each period, animals were sampled immediately following the EE2 exposure and at metamorphic climax (stage 42). Tadpoles exposed to EE2 during mid-metamorphosis were developmentally delayed immediately following exposure and took approximately two weeks longer to reach metamorphic climax. With regards to the feminizing effect of EE2, metamorphs displayed a higher incidence of intersex (17%) and a strong female-biased sex ratio (1:9; M:F) when exposed early in development, while, in comparison, the control group contained no intersex individuals and exhibited a 1:1 M:F sex ratio. This demonstrates that transient early life-stage exposure to estrogen can induce effects on the reproductive organs that persist into the beginning of adult life-stages. Overall this work

provides evidence that there are distinct phases of sensitivity to estrogens, where early versus mid-developmental exposures affect sexual development and rate of metamorphosis, respectively. This has important implications for the understanding of stage-dependent effects of endocrine-disrupting chemicals.

Effects of oil sands waste water on the wood frog (Rana sylvatica). B. Hersikorn[1] and J. E. Smits[1] (PO)

[1]University of Saskatchewan, Saskatoon, SK

Extracting oil from oil sands produces large quantities of waste tailings water reclaimed through formation of wetlands. To understand the impact of these wetlands, a mesocosm study of indigenous amphibians (Wood Frogs) was carried out during the spring and summer, 2006, 2007. In both years, frogs were exposed to wetlands containing oil sands process-affected water (OSPW) and reference water (no OSPW). In year one, there was one site with six experimental trenches, three of which contained OSPW and three contained reference water. Each trench had three enclosures with 50 tadpoles each. On a third OSPW affected site, there were three enclosures, each containing 50 tadpoles. In year two there were 13 sites; six reference and seven OSPW- affected sites, which were classified as old ($\geq$ 8 yrs) or young ($\leq$7 yrs). Four enclosures, with 50 tadpoles each, were placed in each wetland. Growth rate, survival, time to metamorphosis, thyroid hormone concentrations, liver EROD activity, tissue retinol concentrations, and stable isotope analysis are being or have been evaluated. Stable isotopes are being used to track carbon flow from primary production plants, through the food chain, to tadpoles and frogs which represent intermediate and higher trophic levels in reclaimed wetlands. The development and health of native amphibians reflects the sustainability of the different reclamation strategies and details the impacts of OSPW to higher trophic level organisms.

Aquaculture

Session Co-chairs/ Présidents Cheryl Podemski and Les Burridge

The Salmon Aquaculture Dialogue Working Group on chemical inputs from salmon aquaculture. L.E. Burridge[1], J. Weis[2], F. Cabello[3], J. Pizarro[4] and K. Bostick[5] (PL)

[1]Fisheries and Oceans Canada, St. Andrews, NB; [2]Rutgers University, Piscataway, NJ; [3]New York Medical College, Valhalla, NY; [4]Universidad de Santiago de Chile, Santiago, Chile; [5]World Wildlife Fund, Washington, DC

The World Wildlife Fund is facilitating a dialogue on impacts of salmon aquaculture. The Salmon Aquaculture Dialogue is overseen by a steering committee made up of representatives from industry and from environmental groups from Europe, North America and Chile. The goal of the dialogue is to establish the state of knowledge in seven subject areas associated with the industry: benthic impacts, nutrient loading, escapees, chemical inputs, diseases, feeds and social issues. Working groups have been established to prepare summary reports and make recommendations. The chemical inputs working group has summarized the use of chemicals in various jurisdictions (Norway, UK, Canada and Chile), compared regulations and quantities and types of compounds

used, and prepared recommendations for further discussion within the dialogue. The problems associated with fish culture are similar in all jurisdictions, but the magnitude of problems is not and the number of compounds available to the fish farmer varies. Regulations are in place in all jurisdictions that identify the compounds that can be used as antifoulants, antibiotics, parasiticides, anaesthetics and disinfectants. Unfortunately, the requirement to report on chemical use is inconsistent at best, and finding data on what is used and in what quantity is very difficult. The authors will report on the status of chemical use and present some of the recommendations they have put forward to the Salmon Aquaculture Dialogue.

A review of the physiochemical properties of salmonid faeces: Implications for ecosystem-based management. G. Reid[1], L.E. Burridge[2], M. Liutkus[3], S. Robinson[2], T. Lander[2], T. Chopin[1], F. Page[2] and T. Blair[2] (PL)

[1]University of New Brunswick/ Fisheries and Oceans Canada, St. Andrews, NB; [2]Fisheries and Oceans Canada, St. Andrews, NB; [3]University of New Brunswick, Saint John, NB

Cage-based salmonid aquaculture is being considered within ecosystem-based management (EBM) frameworks in many jurisdictions. Organic and inorganic nutrient-loading models that help identify sustainable thresholds of assimilative capacity, and integrated multi-trophic aquaculture (IMTA) as a means to recover 'lost nutrients,' are two methods that facilitate EBM. Faeces make up the vast majority of organic solids exiting salmonid cages, and consequently, biophysical faecal properties have significant implications for IMTA optimization, the dispersal of faecal solids and potentially associated compounds such as therapeutants. Using a mass-balance nutritional approach to calculate the amount and proximate composition of salmonid faeces has been reasonably well validated. However, physical properties and processes can have significant implications on the fate of faeces, and are less well understood. Faecal nutrient content and density will vary with submersion time, and some nutrient leaching may occur in minutes. Indigestible dietary components have the potential to affect faecal 'cohesiveness', dispersal, and consequently 'capture ability' by co-cultured species. Published settling velocity data on salmonid faeces is variable due to differences in fish size, species, rearing systems, collection time, water density, collection method, the mass fraction tested, and diet. Settling data used in salmonid deposition models are very rudimentary, and recent information suggests that such models are highly sensitive to this input. Effective application of models and IMTA practices is further challenged by the lack of information on faecal particle size and the relative proportions of settleable vs. non-settleable mass fractions, specifically in cage environments. Additional implications and research recommendations are discussed.

Environmental impacts of freshwater cage culture: overview of results from the ELA whole-lake experiment. ***C. Podemski[1], R. Rooney[2], D. Findlay[1], K. Mills[1], P. Azevedo[1], M. Kullman[3], K.A.Kidd[3], M. Paterson[1], P. Blanchfield[1], L. Tate,[1] R. Hesslein[1] and M. Wetton[2] (PL)***

[1]Fisheries and Oceans Canada, Winnipeg, MB; [2]University of Manitoba, Winnipeg, MB; [3]University of New Brunswick, Saint John, NB

In 2001, researchers at the Experimental Lakes Area located in northwestern Ontario initiated a whole-lake experiment in order to develop an understanding of how freshwater cage culture of salmonids affects the receiving lake ecosystem. The multidisciplinary group of researchers have been monitoring changes in water chemistry, phytoplankton, zooplankton, sediment chemistry, benthic invertebrates, the utilization of farms wastes by the native food web, and the growth and behaviour of native fishes as well as escapees. The 10T rainbow trout farm is currently in its fifth and final year of fish production. Operation of the farm has resulted in increased annual loading of N and P to the lake by approximately 4x and 15x, respectively. Mean ice-free phytoplankton biomass has increased, largely due to the presence of spring blooms, and we have observed changes to the benthic environment and its inhabitants as well as increased growth of native fishes. Stable isotope analysis indicates widespread usage of farm wastes by native biota. This presentation will provide an overview of results from the first four years of operation of the farm.

A whole-lake experiment to assess impacts of rainbow trout cage culture: Lake trout enhancement? ***K. H. Mills[1], C. L. Podemski[1], P. J. Blanchfield[1], S. M. Chalanchuk[1] and D. J. Allan[2] (PL)***

[1]Fisheries and Oceans Canada, Winnipeg, MB; [2]Limno Tech Enterprises, Winnipeg, MB

Researchers at the Experimental Lakes Area (ELA), northwestern Ontario, are conducting a cage aquaculture experiment to assess its impact on a small oligotrophic lake ecosystem. Approximately 10,000 rainbow trout have been cultured during each of the past four years, 2003-2006, in Lake 260 at the ELA. The purpose of this presentation is to describe changes in the native fish community of Lake 260 during the four years of cage culture. There were few changes in the water chemistry or other lake biota, including fish populations, during the first year of cage culture, but changes started the second year and have continued each subsequent year that rainbow trout were cultured in the lake. The biomass of fish populations in the lake has increased, primarily due to increased abundance of each species except lake trout. Growth and fatness of native lake trout have increased and age of first maturity has decreased, so increased recruitment will likely occur and abundance will increase. After four years of cage culture, there have been few negative impacts on the biota in this lake, and lake trout abundance may become enhanced.

Environmental monitoring of aquaculture in Nova Scotia. M. Tekamp[1] and T. Balch[1] (PO)

[1]Nova Scotia Fisheries and Aquaculture, Halifax, NS

Nova Scotia Fisheries and Aquaculture's Environmental Monitoring Program studies the relationship between aquaculture and the marine environment. Based on a protocol established by government, academia, and industry, monitoring is conducted both on aquaculture leases and at reference stations in the surrounding bay. Monitoring consists of collecting qualitative (video) and quantitative (sediment and water analysis) elements from coastal areas throughout Nova Scotia.

The Environmental Monitoring Program (EMP) follows a risk-based approach that recognizes increased risk requires increased monitoring. All marine sites currently in production are targeted and those that are larger or more intensive are given higher priority. Those sites of potential concern are subject to repeat sampling and, if required, remediation action is implemented.

In just four years, the EMP has taken over 1400 sediment samples from more than 500 stations in some 40 different bays. The EMP is finding that as sites are measured multiple times, in separate seasons, it is possible to observe marine environmental change and act accordingly as responsible environmental managers. Nova Scotia Fisheries and Aquaculture (NSFA) can now measure and compare risk between variables (e.g. finfish vs. shellfish, bay vs. site, active site vs. non-active site). With such extensive baseline data, NSFA can better organize the field component of actual site monitoring by focusing on sites of particular interest.

The EMP is also working on a variety of related projects with both regulatory and scientific partners to continue to expand the knowledge base of the potential environmental impacts of aquaculture on our coastal ecosystems. The EMP has also been communicating the results at presentations to a variety of audiences and has generated interest from across Canada. The feedback is very encouraging, not only on the program itself, but also on the results, which demonstrate low environmental impact of Nova Scotia aquaculture.

Tribute to Scott Brown

Session Co-chairs/ Présidents Wayne Fairchild and Andrea Buckman

An introduction to the Tribute to Scott Brown session. W. Fairchild[1] and A. Buckman[2] (PL)

[1]Fisheries and Oceans Canada, Moncton, NB; [2]Fisheries and Oceans Canada, Victoria, BC

This session was organized as a tribute to the late Scott Brown, who died unexpectedly in June of 2006. We would like to thank the ATW 2007 Organizing Committee for accommodating this session and Karen Kidd for getting the co-chairs involved. Thanks

also go to the presenters of today's talks and all their co-authors, some of whom could be here, while others could not. An acknowledgement and thanks go also to the Environment Canada website and to people who provided pictures for the session. Scott was more often behind the camera than in front of it when it came to pictures.

Scott was a "prairie puddle-jumper" whose work on fish took him to ever bigger puddles, from the Experimental Lakes Area (ELA) to the Great Lakes and the big pond of the North Atlantic Ocean. For those of you who did not know Scott and his work, there will be some interesting presentations here today. For those of you who knew Scott as an associate, colleague, mentor or friend, we hope that today's session can help put in perspective a life taken away too soon, and help in some small way to put the shock of the news from last year behind us. I will go through a brief discussion of Scott's career and accomplishments, but this is in no way an adequate reflection of what it meant to be able to work with Scott or discuss science, or occasionally have a beer or two.

From down on the farm to the international scene, Scott started out working on a family grain farm, driving trucks and working with a maturity beyond his years (or height when it came to reaching the pedals). When he hit science full stride, it was in Geoff Eales' (University of Manitoba) lab working on thyroid metabolism and developing methods still cited and in use. Scott worked his way up at Fisheries and Oceans Canada's Freshwater Institute from summer student to Research Scientist, and then moved in the 1990s to Environment Canada's NWRI, Burlington, and became a Project Chief. He worked nationally and internationally and contributed substantially to the science agenda and regulatory discussions.

Scott's research areas included the effects of priority substances on thyroidal status in fish; priority substance and chemically-induced alterations in functional development, growth and reproduction of fishes; the role and significance of endocrine disruptors (e.g., nonylphenol) in parr-smolt transformation and their subsequent effects on seawater growth and survival in anadromous salmon; among contaminants, micronutrients, gonadal development and early mortality in fishes. Scott had received an Environment Canada Certificate of Recognition for contributions to Northern River Basins Study. His research and scholarship contributed to the basic understanding of mechanisms maintaining homeostasis, development and reproductive physiology in fish.

Scott's Major Affiliations/ Memberships included:

Associate, Graduate Faculty, Department of Zoology, University of Guelph
Honorary Research Associate, Department of Biology, University of New Brunswick
Member, Society of Environmental Toxicology and Chemistry,
Member, International Association for Great Lakes Research
Board of Technical Experts for Great Lakes Fishery Commission –
Early Mortality Task Leader
Delegate, Organization for Economic Cooperation and Development (OECD)
Endocrine Disrupters Testing and Assessment Task Force

Presentations in this session (presenting author in parentheses) covered thyroid hormone status in fish from contaminant effects (Deb MacLatchy) and PCB and temperature effects standpoint (Aaron Fisk); vitamin metabolism (Vince Palace); early mortality syndrome (Jacqueline Arsenault); retinoids and reproduction (Derek Alsop); marine survival of salmon smolts after freshwater exposure to contaminants (Les Burridge); and rounded off with a presentation on Great Lakes Areas of Concern and hydroxylated PCBs (Linda Campbell). Abstracts and a complete author list for each talk are available elsewhere in the proceedings.

On a more personal note from this author (WF), I first met Scott as part of a gathering called FIGS (Freshwater Institute Graduate Students), which provided many thought-provoking science discussions and was also a thinly-veiled excuse to drink beer on Friday afternoons. I also had the privilege of being on the executive of the tongue-in-cheek PWTLSB Society (People Who Think Like Scott Brown), which was of course another excuse to drink beer and talk science. I spent a fair bit of time with Scott and others, planning and writing manuscripts in St. Andrews, NB, where beer was also involved, but with a view of the ocean, and discussions of kids, family and life. Lest this be too much about beer, rest assured that much Timmy's coffee also was consumed on site visits such as our Maritime STP and tour. We talked about EDCs and invertebrates in freshwater, years before I realized Scott was who I needed to call about salmon and EDCs. Our Atlantic salmon smolt collaboration resulted in my most intense day at a conference ever, being questioned by industry for hours, and I was ever so glad to have Scott in the trenches with me.

To me, shifting Scott's viewpoint on something scientific was worth more than many a day's work. Scott was a participant, an active thinker, reader and doer; he was engaged and engaging. I carry with me always his admonition that "the pipes" are the most important aspect of science. No matter how small a thing you are working on, always be aware of how what you are doing fits into the bigger picture.

Thyroid hormone status in fish: Contributions by Scott Brown to understanding contaminant effects. G. Eales[1] and D. MacLatchy[2] (PL)

[1]University of Manitoba, Winnipeg, MB; [2]Wilfrid Laurier University, Waterloo, ON

In fish, thyroid hormones control and integrate key processes such as development, growth, nutrient utilization, and reproduction. Environmental contaminants, including organics (such as PCBs, pesticides and PAHs), metals, and pharmaceuticals have been shown to alter thyroidal status in numerous fish species. For close to twenty years, Scott Brown investigated the effects of anthropogenic activities on thyroid endocrine status in fish. His focus ranged from effects of acidification and metals to PCBs and furans and pesticides, as well as to interactions of xenobiotic effects on thyroid and reproductive endocrine systems. While enhancing our knowledge about contaminant effects, his work also led to a deepening of our understanding of the physiological role of thyroid hormones in fish. Most recently, his interests were focused on determining appropriate

biomarkers in the thyroid hormone cascade for ecotoxicological studies and standardizing test conditions for cause-effect studies linking contaminant exposure, thyroid status, and biological effects. This presentation will provide an overview of Scott Brown's contributions to the field, including a perspective on how his work continues to inform present questions related to contaminant-induced thyroid hormone disruption in fish.

Factors influencing the bioaccumulation of PCBs by fish, and potential interactions with the thyroid axis. A. Fisk[1], A. Buckman[2], V. Palace[3] and S.B. Brown[4] (PL)
[1]University of Windsor, Windsor, ON; [2]Fisheries and Oceans Canada, Sidney, BC; [3]Fisheries and Oceans Canada, Winnipeg, MB; [4]Environment Canada, Burlington, ON
Concern about the influence of PCBs on the thyroid systems of Great Lakes organisms, including humans, has been present for decades. Over the past 20 years, we have carried out a large number of laboratory experiments to quantify the bioaccumulation of PCBs and attempt to determine if PCBs are a factor in thyroid microfollicular hyperplasia observed in Great Lakes salmonids. These experiments have used a number of species, exposure routes, and environmental conditions (e.g., water temperature), and have provided insight into PCB bioaccumulation and biotransformation and the effect of these chemicals on the thyroid axis of fish. Bioaccumulation of PCBs was found to increase with decreasing water temperatures, increase with fish size, and vary with the method of exposure, physical/chemical properties of the PCB congeners, and with the suite of PCBs to which the fish were exposed. In general, the influence of PCBs on the thyroid axis of fish was limited to high PCB exposure concentrations, and, where disruptions were seen (only in specific components of the thyroid system of fish), under specific environmental conditions. Results of these studies suggest that PCBs do not play a significant role in observed thyroid microfollicular hyperplasia observed in Great Lakes salmonids, or that factors (e.g., additional stresses) in addition to PCB exposure and duration of exposure may be responsible for eliciting effects observed in wild fish.

Scott Brown's contribution to our understanding of how PCBs affect vitamin metabolism. V. Palace[1], R. Evans[1], C. Baron[1] and A. Fisk[2] (PL)
[1]Fisheries and Oceans Canada, Winnipeg, MB; [2]University of Windsor, Windsor, ON
By the mid 1990s it was already recognized that polychlorinated biphenyls and other similar coplanar compounds could affect reproduction and growth in exposed organisms. At that time, however, there was a growing recognition that these compounds may mediate some of their effects via disruption of micronutrient metabolism. Scott Brown actively pursued several laboratory and field experiements to examine the mechanisms of altered vitamin metabolism in fish exposed to planar organochlorines. Results from these early studies have been instrumental in guiding ongoing work that has led to a better understanding of vitamin metabolism dynamics.

Early Mortality Syndrome. S. B. Brown[1], J. D. Fitzsimons[2] and J. T. Arsenault[2] (PL)
[1]Environment Canada, Burlington, ON; [2]Fisheries and Oceans Canada, Burlington, ON
Early mortality syndrome (EMS) is the term used to describe mortality affecting early life stages of various salmonid species in the Great Lakes. Highest occurrence of EMS is evident in Lake Michigan and Lake Ontario salmonids. The clinical signs of EMS, which can include loss of equilibrium, swimming in a spiral pattern, lethargy, hyperexcitability, or hemorrhage, culminate in death unless affected fry are given a thiamine treatment. The signs develop just before first feeding, with the timing of onset directly related to egg thiamine concentration. Eggs of affected stocks have very low thiamine levels, and epidemiological studies have linked this with the manifestation of EMS. The exact cause of thiamine deficiency in feral broodstock eggs is unknown. A likely cause of the deficiency is the presence of certain thiamine degrading factors, such as thiaminase found in diet items of the adult salmonids. Salmonids feed mainly on alewife and smelt, which were introduced, respectively, in the Great Lakes in the late 1800s and 1920s. These forage species have high thiaminase activity. EMS prevalence in the Great Lakes has been variable in past years, depending on the lake, species and state of alewife stock. EMS remains a potential threat for the sustainability of the feral Great Lake salmonid stocks. Future EMS research is directed towards establishing a laboratory model for EMS, determining the potential of thiamine-contaminant interactions, the sublethal effects of thiamine deficiency in adults and their offspring, and establishing links between thiamine deficiency and environmental change.

The requirement of retinoids in the reproduction of zebrafish. D. Alsop[1], J. Matsumoto[1], C. Simpson[1], S.B. Brown[2] and G. Van Der Kraak[1] (PL)
[1]University of Guelph, Guelph, ON; [2]Environment Canada, Burlington, ON
The extent to which the retinoids are required in fish reproduction is largely unknown. Retinal is deposited in the egg yolk during oogenesis, as a retinoid source for the future embryo. Retinoic acid (RA) signaling may be active in fish gonads, since the mediators of RA action, the RA receptors (RARs) and retinoid X receptors (RXRs), are present in rainbow trout ovaries and testes. In this study, RT-PCR was used to determine if the enzymes involved in the synthesis and degradation of RA [retinal dehydrogenase 2 (RalDH2) and CYP450RAI (CYP26a), respectively] and the RA receptors (RAR and RXR subtypes) are expressed in the ovaries and testes of zebrafish. Zebrafish were also exposed in vivo to an inhibitor of RalDH2, diethylaminobenzaldehyde (DEAB), for 11 days, and the effects on egg production and fertilization rate were determined. In addition, real time PCR was used to determine the expression of gonadal RalDH2 and CYP26a. A final experiment examined the long-term (130 d) effects of a retinoid-deficient diet on whole body and egg retinoid levels, and the same reproductive endpoints were evaluated. It was shown with RT-PCR that RalDH2, CYP26a, and RA receptors (RAR two subtypes) and RXR (3 subtypes) are expressed in the ovaries and testes. Exposure to DEAB decreased the number of spawned eggs by 95% and altered the expression of RalDH2 and CYP26a in the gonads. Zebrafish fed a retinoid-deficient diet

for 130 days had whole-body retinoids decreased in females and males by 68% and 33%, respectively. During the final 12 days of the exposure, females fed the retinoid-deficient diet produced 71% fewer eggs that contained 78% less retinal than controls. Ovarian expression of RalDH2 and CYP26a was not affected by the deficient diet. These studies have shown that the RA signaling system, including enzymes that synthesize RA (RalDH2) and catabolize RA (CYP26a) and multiple receptor subtypes, are expressed in the zebrafish gonads. Moreover, studies using inhibitors of retinoic acid metabolism or retinoid-deficient diets demonstrate that the retinoid system is essential for the spawning of eggs. The retinoid system, while often overlooked in studies of reproductive biology, has an essential role in normal development and should be considered as possible target mediating the toxicity of some chemicals in the environment.

Potential effects of exposure to contaminants in freshwater on marine survival of Atlantic salmon smolts. L. E. Burridge[1], W. Fairchild[2], J. T. Arsenault[2], D. T. Bennie[3], D. Cotter[4], G. Eales[5], K. Haya[1], D. L. MacLatchy[6], M. Khots[7]; J. P. Sherry[3] and S. B. Brown[3] (PL)

[1]Fisheries and Oceans Canada, St.Andrews, NB; [3]Environment Canada, Burlington, ON; [4]Marine Institute, Newport, Ireland; [5]University of Manitoba, Winnipeg, MB; [6]University of New Brunswick, Saint John, NB, [2]Fisheries and Oceans Canada, Moncton, NB; [7]Long Island, NY

We previously identified relationships between historical applications of a forest insecticide containing 4-nonylphenol (4-NP) and catch data for Atlantic salmon *(Salmo salar)* populations. To test the hypothesis that 4-NP impairs parr-smolt transformation, we exposed Atlantic salmon smolts to pulse doses of water-borne 4-NP (20 $\mu g \cdot L^{-1}$). As positive control for estrogenic responses, we also exposed smolts to estradiol (E2) (100 $ng \cdot L^{-1}$). We monitored smolt response to a seawater challenge and subsequent growth in seawater. We assessed osmoregulatory competance, indicators of energy balance, reproductive steroids, the estrogenic marker vitellogenin, and hormonal regulators of growth (e.g. insulin-like growth factor, thyroid hormones, thyroid hormone deiodinases, and thyroid histology). There were no treatment-related increases in mortality during a seawater challenge soon after exposure. However, growth in seawater was repeatedly impaired in 25-35% of fish from various treatment groups (5% in control). Plasma IGF-1 concentrations were affected by both 4-NP and E2. Smolt growth in the first months at sea and smolt plasma IGF-1 concentration, have been positively related to the rate of subsequent salmonid adult returns. An ocean release field study on the Burrishoole River, Ireland, will be described which suggests that exposure of smolts to 4-NP can impair subsequent seawater survival such that adult returns to the coast are reduced. If these effects are due to steroidogenic potential, then steroidogenic activity stemming from various effluent sources may influence present-day salmon populations. Current research is looking at the effects of pesticides in pulse doses on subsequent smolt survival and growth.

Hydroxylated polychlorinated biphenyls (OH-PCBs) and other halogenated phenolic compounds in fish from Areas of Concern in the Great Lakes . D. Muir[1], C. Darling[1], L. Campbell[2], D. Ueno[3], M. Alaee[1], G. Pacepavicius[1], M. McMaster[1], J. Sherry[1] and S.B. Brown[1] (PL)

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Halogenated phenolic compounds (HPCs) such as hydroxy-PCBs (OH-PCBs), hydroxy-PBDEs (OH-PBDEs), triclosan, and other chloro- and bromophenols are of interest as possible thyroidogenic chemicals. There has been a limited amount of research on these chemicals in the Great Lakes region. Typically, higher concentrations of HPCs are seen in water near municipal waste outfalls, but overall, little is known about the levels of exposure of fish and wildlife. In this study, HPCs were determined (OH-PCBs, pentachlorophenol PCP, OH-BPDEs, and triclosan) in fish plasma from five Areas of Concern and their associated reference sites on Lakes Ontario and Erie to assess exposure and possible links to biomarkers of thyroid activity. Most of the work was conducted using brown bullheads *(A. nebulosus),* a bottom feeder with known high exposure to PCBs. Plasma was extracted with MTBE/hexane, partitioned with KOH (0.5 M) and the neutral compounds (PCBs, PBDEs, etc.) removed in the organic phase. The HPCs were ionized and isolated from the aqueous phase, then methylated with diazomethane and analysed by gas chromatography– high resolution mass spectrometry (GC-HRMS). OH-PCBs, triclosan, and PCP were detected in all fish analysed. Triclosan was the most prominent individual HPC in bullhead plasma (1-10 $ng \cdot g^{-1}$ range) and was present at higher concentrations near AOCs than at reference sites. The majority of OH-PCBs detected by GC-HRMS were unidentified. The concentrations of OH-PCBs and OH-PBDEs in fish appear to be affected by prevailing water temperatures, thus seem to be derived from CYP processes rather than uptake from water or food.

Omics (genomics, proteomics, metabolomics)

Session Co-chairs/ Présidents Caren Helbing and Graham van Aggelen

Development and application of toxicogenomics to aquatic toxicology. H. Osachoff[1], J. Bruno[1], R. Skirrow[1] and G. van Aggelen[1] (PL)

[1]Environment Canada, North Vancouver, BC

Environment Canada's Water, Science and Technology Branch, Pacific Environmental Science Centre (PESC) has been actively involved in the field of toxicogenomics for over seven years. During this time the laboratory has built expertise and capacity for conducting large-scale toxicogenomic studies in freshwater and marine environments. With collaborating partners, we have designed a targeted Rainbow trout gene array and conducted toxicogenomic investigations into pure chemicals and complex mixtures. Presentation will focus on the application of our genomic activities and insight into the future direction of toxicogenomics at the Centre.

An update on fish and chips and the utility of these and other genomic tools. J. Bruno[1], R. Skirrow[1], H. Osachoff[1] and G. Van Aggelen[1] (PL)

[1]Environment Canada, North Vancouver, BC

For the last eight years, the Toxicology Section at the Pacific Environmental Science Centre (Environment Canada) has been developing and utilizing toxicogenomic tools to evaluate the molecular-level impact of effluents on sentinel aquatic species. Toxicogenomics is a field of toxicology utilizing molecular techniques to identify gene expression changes resulting from material exposures. Toxicological testing, whether laboratory or field based, has been and continues to be an integral toolset for use in evaluating the potential of a toxicant to cause some measurable effect. Traditional endpoint measurements such as death, growth or reproduction were previously the chief means of gauging the deleteriousness of an effluent; however, while many of the effluents of historical concern are no longer acutely toxic, toxicological effects, such as feminization and intersex fish and amphibian, have been observed in fish exposed in the wild. Integrating genomics into the standardized battery of toxicity test methods generates molecular data that can help determine the extent and ecological relevance of exposure. This added information may provide an early indicator of subsequent population or community level effects. The application of genomic tools such as microarrays and real time PCR in toxicology testing will be discussed in the context of data obtained from several exposure studies.

Gene expression of multixenobiotic resistance proteins, p-glycoprotein and mpr2, in the Antarctic emerald rockcod (Trematomus bernacchii). S. Zucchi[1] (PL)

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We examined the gene expression of P-glycoprotein (P-gp/MDR1/ABCB1) and Multidrug Resistance-associated Protein 2 (MRP2/ABCC2) in *Trematomus bernacchii*, the Antarctic emerald rockcod, experimentally exposed to organic contaminants and heavy metals. P-gp and MRP2, ATP-binding cassette (ABC) proteins, are xenobiotic transporters which respectively facilitate the transmembrane efflux of moderately hydrophobic substrates and phase II metabolite glutathione conjugates. We analyzed *T. bernacchii* because this widely distributed benthic feeder is naturally exposed to high levels of cadmium from the local Antarctic geology and is a key sentinel species for monitoring the Antarctic ecosystem. Our main interest is to assess the sensitivity of *T. bernacchii* to anthropogenic and natural chemicals. In contaminated exposed fish, we investigated the expression of the two transporters who play a role in absorption, disposition and elimination of a wide variety of xenobiotics. We evaluated fish (N=8) exposed by intraperitoneal injection to either a single contaminant or pairs of contaminants from the following list: benzo[a]pyrene, 2,3,7,8-tetrachlorodibenzo-p-dioxin, cadmium, copper and lead. Partial sequences of P-gp (410 nucleotides) and MRP2 (750 nucleotides) were obtained and used to design primers to assess hepatic P-gp and MRP2 gene expression by quantitative reverse transcriptase-polymerase chain reaction

(qRT-PCR) in exposed fish. To determine whether P-gp and MRP2 hepatic gene expression reflects protein expression in the liver, we evaluated protein levels by Western blot. This study provides a preliminary characterization of genes involved in cellular defense mechanisms in the key sentinel species *T. bernacchii* and evaluates whether anthropogenic and natural compounds could modulate P-gp and MRP2 expression.

In vitro exposures of Solea solea microsomes and hepatocytes to benzo[a]pyrene and fluoranthene: metabolite identification and genotoxic effects. N. Wessel[1], F. Akcha[1], M. Le Dû-Lacoste[2], K. Pichavant[3], H. Budzinski[2], T. Burgeot[1]. (PL)

[1]IFREMER Nantes, France ,[2]Université de Bourdeaux,Bourdeaux, France, [3]Université de Bretagne Occidentale, Brest, France

This study is part of a PhD project on the bioactivation and the genotoxic effects of PAHs in juveniles of the flatfish species, *Solea solea*. Two PAHs were selected for this study. Benzo[a]pyrene (BaP) was selected as a model genotoxicant because of its well-described bioactivation pathways and genotoxicity. Fluoranthene was chosen for different criteria: 1) it is one of the most abundant PAH in the marine environment, 2) there is very poor data concerning its metabolism and genotoxicity, 3) its metabolism is thought to be different from that of BaP with respect to structure and bioaccumulation. In a first set of experiments, microsomal incubations were performed for each PAH for the analysis of the metabolites produced by liquid chromatography coupled to mass spectrometry. In parallel, data on the genotoxicity of the parent compounds were obtained by exposing primo-cultures of sole hepatocytes. Genotoxicity was assessed by measuring DNA strand breakage (Alkaline comet assay) and the oxidised DNA base, 8-oxodGuo (HPLC coupled to electrochemical detection).

Lipid- and cholesterol-lowering drugs and an anti-depressant modulate multiple pathways within steroidogenesis and reproduction in fish. T. Moon[1], C. Mimeault[1], A. Woodhouse[1], C. Cameron[1], C. Estey[1], L. Bullock[1], J. Mennigen[1] and V. L. Trudeau[1] (PL)

[1]University of Ottawa, Ottawa, ON

Human pharmaceuticals are found in the aquatic environment at concentrations in the nanogram to microgram·L^{-1} range. These drugs are designed to act on receptors and/or signalling systems, and in so doing they potentially disrupt gene transcription in non-target aquatic species. Gemfibrozil (GEM), a fibrate drug, decreases testosterone levels in goldfish. This effect may be mediated through changes in proteins and enzymes within steroidogenesis by both peroxisomal proliferator-activated receptors (PPARs) and non-PPAR pathways. However, a statin drug (cerivastatin) acts in vitro and vivo to inhibit the first committed enzyme in cholesterol biosynthesis in trout; this inhibition may be directly on the enzyme rather than at the transcript level. Using a goldfish-carp cDNA array, we have demonstrated that fluoxetine, a serotonin-reuptake inhibitor, affects a

number of critical genes, but specifically down-regulates isotocin, a neuropeptide that modulates spawning and social behaviors in fish. These changes are correlated with changes in egg production in some fish species. These studies demonstrate that the human pharmaceuticals tested act in fish as they do in humans, but whether they affect the fitness of these species is yet to be established. Research supported by NSERC of Canada Strategic and Discovery Grant Programs, Ontario BEST in Science, and Pfizer Inc.

Toxicogenomic research using salmonids and zebrafish as model organisms. M. Rise[1] and M. Carvan[2] (PL)

[1]Memorial University of Newfoundland, St. John's, NL; [2]University of Wisconsin, Milwaukee, WI

Environmental pollutants are serious threats to aquatic vertebrates. We utilize genomic techniques, such as suppression subtractive hybridization (SSH) cDNA library construction, quantitative reverse transcription – polymerase chain reaction (QPCR), and DNA microarray hybridization, to study salmonid and zebrafish gene expression responses to toxicants such as heavy metals, pesticides, and dioxins. There are excellent genomic resources available for Atlantic salmon, rainbow trout, and zebrafish. We use ~3500-gene (3.5K) and 16K salmonidcDNAmicroarrays from the consortium for Genomic Research on All Salmonid Project (cGRASP) and QPCR to identify and validate rainbow trout genes responsive to waterborne exposures to the organophosphate pesticide (OP) chlorpyrifos. Candidate informative genes validated by QPCR form a suite of chlorpyrifos-responsive molecular biomarkers that may be used to evaluate the biological impacts of OPs on feral and cultured fish populations. In addition, we use custom-built 8K cDNAmicroarrays and QPCR to study gene expression changes occurring in zebrafish embryos exposed to varying concentrations of methylmercury via the maternal diet. Candidate mercury-responsive genes identified in this work have functions associated with neurological structure and function, mitochondrial function, and immune system function. Current work involves parallel exposures of salmonids and zebrafish to toxicants (mercury, dioxins) and the use of genomic techniques to identify conserved and lineage-specific genes involved in fish responses to these environmental stressors.

Application of proteomic profiles of the zebrafish (<u>Danio rerio</u>) gill and liver to toxicology. G. Goss[1] (PL)

[1]University of Alberta, Edmonton, AB

Using high-throughput mass spectrometer-based methods (RPLC-ESI MS/MS) we have identified over 4500 proteins in the zebrafish gill and over 7000 proteins in the zebrafish liver. Proteins were characterized according to their cellular process, their molecular function, and their subcellular location. Numerous proteins implicated in physiological responses to environmental stressors and toxicants were identified and we are currently developing new labeling techniques to quantify changes in the proteome in response to

these stimuli. The goal of this study is to further develop the zebrafish as a model system for basic physiological research and for aquatic toxicology. Quantifying specific changes in the zebrafish proteome will be a powerful tool in a variety of fields, including identifying biomarkers for emerging toxicants and novel manufactured compounds, understanding responses to natural physiological stimuli (e.g. hypoxia, hypercarbia) and uncovering developmental processes.

Genomics and proteomics approaches for the detection of perturbations in thyroid hormone action in amphibian sentinels. C. Helbing[1], D. Domanski[1], M. Gunderson[1], L. Ji[1], S. Maher[1] and N. Veldhoen[1] (PL)

[1]University of Victoria, Victoria, BC

Despite the number of genome sequences available, only a tiny fraction of the diversity of life on our planet is represented. Sentinel species for which substantial toxicological data exist are largely underrepresented in the genomics and proteomics arena. This is certainly the case for frogs and toads. Nevertheless, genomics and proteomics approaches are still applicable to these species. Thyroid hormones (THs) are important in the regulation of growth, development and metabolism in vertebrates, and there is growing concern that environmental contaminants may disrupt TH action. In frogs, THs are essential for metamorphosis of the tadpole into a frog. Disruption of TH action can be detected by alterations in the transcriptome and proteome prior to overt morphological responses. We have developed a cDNA array that was specifically designed to allow for cross-species hybridization and show that it is effective on species with limited genomic information. We have also developed and applied an approach involving non-lethal tail fin biopsy procedure combined with gene expression biomarker analyses to detect these changes. These biopsies allow for a repeated measures experimental design and have applicability in field sampling of threatened species. In combination with proteomic endpoints, gene expression screens have great potential for augmenting our ability to detect disruption of TH action.

Endocrine Disruption

Session Co-chairs/ Présidents Thijs Bosker and Deb MacLatchy

A comparison of the occurrence of tributyltin-induced imposex in dogwhelks (Nucella lapillus) in Halifax Harbour (1995-2006). C. Kendall[1] and S. Bard[1] (PL)

[1]Dalhousie University, Halifax, NS

Tributyltin (TBT) is an anthropogenically introduced contaminant chemical compound found in Halifax Harbour. The primary source of this chemical compound is anti-fouling paints formerly used heavily by the shipping industry to prevent the growth of barnacles and other marine species on the hulls of the ships, whose use was partially banned in 1989. The presence of this compound can induce imposex, the imposition of male reproductive organs over the female reproductive system, in dogwelks *(Nucella lapillus)*

at levels as low as 1 $ng \cdot L^{-1}$. The dogwhelk can thus be used as a bio-indicator of TBT contamination. The present study surveyed the imposex levels of dogwhelks at sites throughout Halifax Harbour to determine the presence of TBT contamination. The data were compared to baseline data collected in 1995 to determine temporal changes in TBT contamination in terms of dogwhelk imposex. It was found in the present study that levels of imposex frequency have not decreased significantly in the area. However, the mean female penis length (FPL), and the relative penis size index (RPSI), measurements indicating the severity of imposex, showed a significant decrease from 1995 levels. The results of this study indicate that the partial ban on TBT use in 1989 was effective at reducing imposex in areas of small-boat marinas. There remain, however, continued inputs of TBT from current shipping traffic and persistent TBT deposited in sediments. Complete recovery may be decades after a complete ban on TBT paints in the shipping industry takes effect in 2008.

Bioaccumulation of the synthetic hormone ethinylestradiol in benthic invertebrates, via water and sediment exposure. È. Dussault[1], V. Balakrishnan[2], U. Borgmann[2], K. Solomon and P. Sibley (PL)

[1]University of Guelph, Guelph, ON; [2]Environment Canada, Burlington, ON

Environmental exposure to the synthetic hormone ethinylestradiol (EE2) has received considerable attention in the last two decades, during which its estrogenic effects on the reproductive system of aquatic vertebrates were uncovered. In contrast, effects on aquatic invertebrates are poorly documented, despite their important role and contribution to ecosystem structure and diversity. Moreover, studies investigating effects on benthic invertebrates, which can be exposed to pollutants from both water and sediment contamination, are rare, and potential risks to benthic communities are largely unknown. Previous investigations revealed that the midge *Chironomus tentans* and the freshwater amphipod *Hyalella azteca* are somewhat tolerant to EE2 (LC_{50} ~ 1 $mg.L^{-1}$ in chronic toxicity tests). Monitoring studies have, however, detected low levels of EE2 in aquatic systems, implying chronic, sublethal exposure. Moreover, the relative insensitivity of both *C. tentans* and *H. azteca,* and the physico-chemical properties of EE2 suggest that this compound could bioaccumulate in benthic invertebrates, thereby representing a notable source of contamination to their vertebrate predators. In this study, we investigated the bioaccumulation of EE2 in *Chironomus tentans* and *Hyalella azteca* during a 3-week exposure. The importance of the exposure medium was investigated through a comparison of water and spiked sediment exposure. Our results show that benthic invertebrates accumulated concentration-dependent levels of EE2 from waterborne exposure. Exposure from spiked sediments suggested negligible uptake from the sediments in *Hyalella azteca*. In constrast, *C. tentans* accumulated more EE2 than predicted by water-only exposure, suggesting potential differences between the two species. Results will be discussed in the context of their potential environmental impacts

Gene expression and histological structure as biomarkers of chemical exposure in Japanese medaka. A. Tompsett[1], J. Park[2], X. Zhang[2], M. Hecker[3], P. Jones[1], J. Newsted[3] and J. Giesy[1] (PL)

[1]University of Saskatchewan, Saskatoon, SK; [2]Michigan State University, East Lansing, MI; [3]Entrix, Inc, East Lansing, MI

Genomic and proteomic methods, such as RT-PCR and 2D-PAGE gels, are very sensitive to gross changes but are not able to detect spatial changes in expression or offer cellular resolution. To better understand the subtle effects of chemicals, a dual biomarker system that utilizes whole mount in situ hybridization (ISH) to detect spatial changes in gene expression at the cellular level and basic histological techniques to detect changes in tissue morphology has been developed. To validate the system, four-month-old Japanese medaka *(Oryzias latipes)* were exposed to 1, 10, or 100 μg fadrozole·L^{-1} for 7 d. Fadrozole is a potent inhibitor of aromatase (CYP19), the enzyme that aromatizes testosterone to estradiol. Using ISH with radio-labeled RNA probes, CYP19a mRNA was detected in different tissues. Fadrozole tended to increase expression of CYP19a in ovaries, as well as numbers of early stage oocytes, while there were no observable effects on testes. Due to the relatively low resolution of this ISH method, it was not possible to definitively evaluate the data, especially in male fish. As a result, new methods using fluorescently labeled probes are being investigated to improve sensitivity. The combinatory approach of ISH and histological examination provided a better understanding of the effects of fadrozole than either of the methods alone.

Determination of masculinization of three-spine stickleback (Gasterosteus aculeatus) exposed to a pulp mill effluent. C. Wartman[1], N. Hogan[2], M. Hewitt[3], M. McMaster[3] and M. van den Heuvel[2] (PL)

[1]Atlantic Veterinary College, Charlottetown, PE; [2]University of Prince Edward Island, Charlottetown, PE; [3]Environment Canada, Burlington, ON

Many studies have demonstrated that pulp and paper effluents cause subtle reproductive changes in fishes, including masculinization. The three-spine stickleback *(Gasterosteus aculeatus)* has a well characterized gene product of androgens called spiggin and, as such, has been proposed as a model species to study the potential androgenic effects of anthropogenic compounds. Studies have shown that exposure to pulp and paper mill effluent can elicit masculinization of female fish, resulting in male biased sex ratios and induction of spiggin in this species. The aim of this study is to determine the androgenic potency of a bleached kraft pulp mill effluent through the measurement of functional, biochemical and molecular endpoints related to reproduction in the three-spine stickleback. Fish were exposed to effluent at 0, 1, 10, 100% in a flow-through system (50% replacement over 24 hours) for 7 and 21 days in duplicate tanks using brackish (5 ppt) water. A separate static-renewal exposure to model compounds was conducted and data were used for comparison. Eight to ten females were sampled per treatment per period. There was a statistically significant increase in posterior kidney epithelial cell

height at the highest dose, which paralleled a non-significant trend toward larger kidneys in females. There were no obvious male secondary sex characteristics observed in the exposed females. Other endpoints that will be discussed include the expression of kidney spiggin mRNA and in vitro gonadal steroid production. In vivo measures will be compared to estrogenic and androgenic receptor binding and enzyme inhibition endpoints elicited by effluent extracts.

Pulp and paper mill effluent treatments have differential endocrine disruption effects in rainbow trout. R. Orrego[1], J. Guchardi[1], R. Krause[1], L. Roti[1], V. Hernandez[2], J. Armour[1], M. Ganeshakumar[1] and D. Holdway[1] (PL)

[1]University of Ontario Institute of Technology, Oshawa, ON; [2]University of Concepcion, Concepcion, Chile

Endocrine disruption effects due to pulp and paper mill effluents involving different industrial procedures and effluent treatments (specific extracts obtained from non-treated, primary and secondary treated pulp and paper mill effluents) were evaluated using hatchery-reared immature triploid rainbow trout (*Oncorhynchus mykiss*) in a fish pulse-exposure toxicity experiment. The protocol involved the use of intra-peritoneal injection, corrected for individual fish weight and utilizing previously determined dose information, and also included several laboratory standards (steroidal hormones and phytosterols). Multiple biomarkers at different levels of biological organization (molecular, cellular, tissue and individual organism level) were analyzed. Previous results indicated that non-significant changes were observed in the individual physiological indices represented by condition factor and liver somatic index during different sampling times. Significant induction of the cytochrome P4501A1 was observed between different effluent treatments and experimental controls. Significant endocrine-disrupting effects (reproductive level) were observed in all effluent treatments involving significant increments in gonadal somatic index and plasma vitellogenin (Vtg) levels. There were significant differences in Vtg, indicating a decrease of the endocrine effect due to the effluent treatment. These results will be confirmed by immunohistochemical analysis of Vtg in the fish gonads.

Assessment of biotreatment for reducing the effects of pulp and paper mill effluents on fish reproduction in laboratory tests. T. Kovacs[1], P. Martel and V. Bérubé[2] (PL)

[1]Forest Products Innovation Paprican, Pointe Claire, QC; [2]Quebec Forest Industry Council, QC

Pulp and paper mill effluents have been reported to cause changes in reproductive indicators of fish. We examined the performance of biotreatment plants in abating effects on fish reproduction. A bleached kraft mill effluent (BKME) treated in an aerated lagoon and a thermomechanical pulp mill effluent (TMPE) treated by aerobic sludge in a sequential batch reactor were selected for study. Mature fathead minnows *(Pimephales promelas)* were exposed to effluents before and after biotreatment under continuous

renewal conditions for 21 days. Egg production, fertilization, and hatching were monitored daily, while morphometric parameters (length, weight, gonad size), secondary sexual characteristics, and steroid hormone and vitellogenin levels were measured at the end of the exposure. The effluent from both mills before biotreatment impaired the reproductive capacity of minnows at concentrations of 10% and 20% v/v. Exposure to biotreated effluents from both mills at concentrations of 2%, 10%, 20% and 40% v/v caused no significant differences in overall reproductive capacity of minnows as compared to controls. These results indicate that biotreatment can significantly improve the quality of BKME and TMPE with respect to the reproductive capacity of fish in laboratory tests.

Thermomechanical pulp mill effluent effects on reproduction in mummichog (Fundulus heteroclitus). T. Bosker[1], K. Munkittrick[1], M. Hewitt[2] and D. MacLatchy[3] (PL)

[1]University of New Brunswick, Saint John, NB; [2]Environment Canada, Burlington, ON; [3]Wilfrid Laurier University, Waterloo, ON

Using an investigation of cause (IOC) approach, we studied the effect of the effluent of Irving Paper, Ltd. of Saint John, NB (a thermomechanical pulp mill) on the reproductive status of the mummichog (*Fundulus heteroclitus*), an endemic fish species on the east coast of Canada. The IOC approach uses toxicity source evaluation and toxicity identification evaluation to identify waste stream sources and compounds causing effects of interest. Initial experiments were conducted to confirm the potential impact of the final effluent on fish reproduction. Endpoints analyzed included gonad size, body size, liver size, plasma reproductive endocrine status, egg production and stage of egg development. Relative to reference, no significant differences in the endpoints were observed in fish exposed to 1, 10 and 100% final treated effluent. These data indicate a low potential for an effect of final effluent on the environment. However, there was high variability in the gonad size and endocrine endpoints. Present studies are focusing on reducing variability by improving standardization of the adult mummichog reproduction test and determining adequate power for key endpoints.

A comparison of mummichog (Fundulus heteroclitus) and fathead minnow (Pimephales promelas) reproductive tests as indicators of exposure to pulp mill effluents. S. Melvin[1] and D. MacLatchy[2] (PL)

[1]University of New Brunswick, Saint John, NB; [2]Wilfrid Laurier University, Waterloo, ON

Fish downstream of some Canadian pulp and paper mills show a pattern of "metabolic disruption"—large livers, high condition and small gonads. Studies are underway to determine if adult fish reproductive tests can be used to determine the mechanism of reproductive dysfunction and the sources and identity of contaminants. Challenges include determining whether differences among studies are due to differences in effluent quality among mills or physiological differences among fish species. This study examined the reproductive responses of the fathead minnow *(Pimephales promelas)* and mummichog *(Fundulus heteroclitus)* under controlled laboratory conditions to effluent

from a bleached kraft pulp mill in Saint John, NB. Effluent concentrations for both species were simultaneously maintained at 0, 3, 10, and 30%, and egg production, time to hatch, and offspring survival were the primary endpoints. Sex steroids were measured after in vitro incubations of the gonads, as well as full body steroid levels, to determine if changes in reproduction were mediated by changes in the endocrine system. These studies will help identify the significance of species differences for investigation of cause studies.

Determining biologically active components in kraft mill chemical recovery condensates. C. Milestone[1], V. Trudeau[2], D. MacLatchy[3] and M. Hewitt[4] (PL)

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Previously, the condensate streams from the chemical recovery process in a kraft pulp and paper mill have been identified as depressing sex steroids in mummichog. Using solid phase extraction (SPE), a fractionation regime using two SPE catridges in series was developed, with one particular eluted fraction (SPE2) being identified as containing the majority of biological activity. This fraction was then further separated using preparative scale reverse phase-HPLC. Unfortunately, exposure of HPLC separated fractions to mummichog showed little to no biological effect. Recent work has been to firstly ascertain why biological activity was lost in sample preparation and fractionation using preparative HPLC. It was determined that the active compounds were being lost during solvent evaporation (water and acetonitrile) of samples in preparation for fish exposure studies. Experiments showed that compounds previously identified in the stream were volatilised before all water was evaporated. Because of this, the fractionation procedure of the biologically active SPE2 fraction has been refined. The new regime entails using non-aqueous solvents of decreasing polarity to elute straight from the SPE2 cartridge. These fractions have been tested using both in-vivo (mummichog) bioassays and in-vitro (GABA) tests for neuroendocrine effects.

Fate of aromatic compounds in the mud snail and wrinkle whelk. A. Parsons[1], J. Hellou[2], K. Campbell[1] and M. Quilliam[3] (PO)

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Some xenobiotics, such as pharmaceuticals and other phenolic compounds, can mimic the endogenous estrogen 17β-estradiol (E2) and their uptake may lead to detrimental effects within aquatic life. The abundant priority pollutants polycyclic aromatic hydrocarbons are also potentially estrogenic due to their reactivity within organisms. Underutilized species such as the large offshore snail *Neptunea lyrata decemcostata* (wrinkle whelk) are becoming attractive for the fishing industry. The synthetic compounds bisphenol-A (BPA), butylated hydroxytoluene (BHT), and 17α-ethynylestradiol (EE2), the anthropogenic contaminants pyrene, phenanthrene, and fluorene, and E2 were used in

water exposures involving two snail species. Our interest was to examine the ability of the smaller mud snail *Ilyanassa obsoleta* and larger wrinkle whelk to biotransform these aromatic compounds. Three surrogate standards representative of a wide range of chemical polarities were used to delineate the best recoveries in the analytical approach, since the fate of the tested aromatic compounds was unknown. In a first step, samples were analyzed by high performance liquid chromatography (HPLC) with ultra-violet and fluorescence detectors. This was followed by liquid chromatography-mass spectrometry (LC-MS) to identify metabolites.

The effects of waste water treatment plant effluent and agricultural runoff on the reproductive status of fathead minnow (Pimephales promelas) in southern Alberta. E. Nelson[1], X. Shi[1], H. Habibi[1], A. Hontela[2] and N. Kromrey[2] (PO)

[1]University of Calgary, Calgary, AB; [2]University of Lethbridge, Lethbridge, AB

Pharmaceuticals and pesticides have been detected in the rivers and irrigation canals of Southern Alberta, a semi-arid region with irrigation-dependent crop production, intensive livestock operations, as well as a growing human population. The objective of this study was to investigate whether fish exposed to effluents from wastewater treatment plants (WWTP) and feedlots have reproductive anomalies, and if there are interactions between chemicals and season. The fathead minnow, a sentinel species, was sampled in three canals as well as upstream and downstream of WWTPs in the Oldman and South Saskatchewan rivers in 2006. Biochemical and morphological endpoints were measured to characterize the reproductive status. Histology of gonads was used to determine sex, gonadal maturity and intersex; sex ratios were skewed toward females at most sites. Liver vitellogenin, a biomarker of exposure to estrogen mimics, was analyzed using quantitative RT-PCR and was elevated in fish from some downstream sites. AChE activity, a biomarker of exposure to organophosphate pesticides, was measured in the head and was lower in the most impacted canal but not at river sites downstream from WWTPs. Sampling is in progress for 2007 field season. Preliminary results indicate fathead minnows in Southern Alberta are impacted by anthropogenic chemicals. (Funded by CWN).

Reproductive effects of sewage effluent and fluoxetine on female zebrafish. A. Lister[1], J. Van Zwol[1] and G. Van Der Kraak[1] (PO)

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Fluoxetine is a widely prescribed anti-depressant drug that acts as a selective serotonin reuptake inhibitor (SSRI). It is the active ingredient in the drug Prozac. Recently, fluoxetine has been measured in sewage effluents and surface waters, and detectable levels of fluoxetine have been measured in tissues of fish residing within sewage effluent contaminated streams. The objective of our study was to investigate the effects of fluoxetine (0.32, 3.2, or 32 $\mu g \cdot L^{-1}$) and sewage effluent (1, 10, or 50%) on the reproduction of breeding groups of sexually-mature zebrafish using a 7-day exposure.

Two other groups were included: a solvent control group and a group exposed to 10 ng·L^{-1} ethynyl estradiol. Each treatment group had three replicates. Eggs were collected daily during the exposure and ovarian tissues were analyzed for levels of estradiol and gene expression. Our results indicate that the highest doses of both fluoxetine and sewage effluent decreased egg production significantly compared with the control group, but gonadosomatic indices were not affected by treatments. Ovarian estradiol levels were depressed in fluoxetine-treated fish, but not in fish exposed to sewage effluents. In order to elucidate the mechanism by which these contaminants disrupted ovarian functioning, we measured the mRNA expression of several genes involved in ovarian steroidogenesis and arachidonic acid production. We believe that fluoxetine and sewage effluents exert their effects differently within the steroid biosynthetic pathway, as demonstrated by the varying patterns of gene expression. Of the several genes examined, Aromatase-A was depressed by fluoxetine treatment. We are investigating the hypothesis that fluoxetine exerts its effects at the level of the pituitary, and subsequently alters ovarian reproduction.

***Does BFR 47 affect in vitro steroidogenesis in fish gonadal tissue?* L. Peters[1], K. Pleskach[2], V. P. Palace[2] and G. T. Tomy[2] (PO)**

[1]*University of Manitoba, Winnipeg, MB;* [2]*Fisheries and Oceans Canada, Winnipeg, MB*

Polybrominated diphenyl ether (PBDE) flame-retardants are lipophilic persistent organic compounds used in manufacturing of plastics, electronic equipment, polyurethane foam and textile materials. PBDEs have become an increasingly important environmental problem due to their ability to bioaccumulate and biomagnify. Since the late 1970's, substantial increases of these compounds have been measured in wildlife and human adipose tissue. Recent studies have focused on the potential endocrine-disrupting properties of PBDEs, which include effects on thyroid hormones and gonadal tissue steroidogenesis. Previous *in vitro* studies that have explored the influence of PBDEs on reproductive hormone production, cell proliferation and apoptosis have focused strictly on mammalian models. Recent work has suggested that fecundity and gonadal steroidogenesis may also be affected in fish exposed to PBDEs. As the predominant congener found in fish, the potential effects of BDE 47 on *in vitro* ovarian steroidogenesis were examined in two species of fish.

***Do human pharmaceuticals found in the aquatic environment affect fish species?* T. Moon[1], C. Estey[1], L. Bullock[1], A. Woodhouse[1], C. Cameron[1], C. Mimeault[1] and V. L. Trudeau[1] (PO)**

[1]*University of Ottawa, Ottawa, ON*

Many human pharmaceuticals are now reported post waste water treatment plant and in surface waters at ng·L^{-1} to mg·L^{-1} concentrations. Our labs investigate the effects of lipid- and cholesterol-lowering drugs on the stress and reproductive axes of model fish species. Gemfibrozil (GEM), a fibrate drug, reduces plasma triglycerides, challenges the hepatic antioxidant system and reduces plasma testosterone in goldfish. Specifically StAR (Steroid Acute Regulatory Protein) and peroxisomal proliferator-activated receptor (PPAR) gene transcription are modified by GEM but on a seasonal basis. In addition, other fibrates alter in vitro ACTH-induced cortisol secretion from head kidney cells in

rainbow trout. These fibrate drugs appear to act both on enzymes within steroidogenesis and at steps prior to this pathway. Cholesterol-lowering drugs in trout alter the first committed enzyme in cholesterol synthesis as in mammals and modify development in zebrafish. Thus aquatic species are affected by human drugs in ways similar to those found in mammals. Research supported by the Natural Sciences and Engineering Research Council of Canada.

Differential effects of environmental chemicals and selected pharmaceuticals on aromatase activity. ***E. Higley[1], M. Hecker[2], J. Newsted[2] and J. Giesy[1] (PO)***
[1]University of Saskatchewan, Saskatoon, SK; [2]Entrix, Inc, Saskatoon, SK

Due to concerns about potential disruption of endocrine processes by chemicals, the role of aromatase in the growth and reproduction has been investigated. In the current study, seven chemicals (atrazine, prometon, ketoconazole, aminoglutethimide, fadrozole, prochloraz and forskolin) with different primary modes of action on steroidogenic systems were tested to determine their differential effects on aromatase activity (AA) as measured by the tritium release assay in H295R adrenal carcinoma cells. Three studies were conducted: (A) Cells were exposed to each chemical for 48h and aromatase was evaluated without addition of chemicals to the assay; (B) Cells were exposed as in A but the chemical was also added to the aromatase reaction; and (C) the chemicals were added to unexposed cells during the assay only. Atrazine and forskolin increased aromatase activity when cells were exposed prior to the initiation of the assay, but there was no change in aromatase activity when these compounds were added directly to the enzymatic assay. Prometon and aminoglutethimide increased aromatase activity after exposure for 48 hours but decreased aromatase activity when cells were exposed directly in the assay. Exposure to fadrozole and prochloraz of up to 0.1μM resulted in an increase of aromatase activity, but at greater concentrations enzyme activities gradually decreased. Direct addition of fadrozole and prochloraz to the assay resulted in decreased aromatase activity for all doses tested. Aromatase enzyme activity was increased by over 12-fold in cells exposed to ketoconazole. However, when ketoconazole was added directly to the assay there was a slight increase in aromatase, but at greater concentrations aromatase enzyme activities started to decrease again. The chemicals tested could be grouped into three categories based on their specific interaction with aromatase activity in H295R cells.

Use of Otoliths and Other Calcified Structure to Resolve Useful Information on Fishes

Session Co-chairs/ Présidents Dean Fitzgerald and Martha Jones

Selenium exposure histories derived from analysis of selenium in otoliths of rainbow trout* (Oncorhynchus mykiss) *and brook trout* (Salvelinus fontinalis) *from an area impacted by coal mining in Alberta's eastern slopes. C. LeVasseur[1], G. Sterling[2], N. Halden[1] and V. Palace[3] (PL)

[1]University of Manitoba, Winnipeg, MB; [2]Alberta Sustainable Resource Development, Edson, AB; [3]Fisheries and Oceans Canada, Winnipeg, MB

Examining the potential effects of contaminants on wild fish is often limited by the ability to determine if fish are indeed exposed to the effluent being evaluated. Measuring contaminants in muscle or visceral tissues of the fish can useful, but depuration, metabolic transformation and mobilization of contaminants among tissues dictates that these measures are only indicative of recent exposure. Otolith, inner ear bones in fish, are metabolically stable and have been used to age fish because of the regular accumulation of calcium carbonate in daily and annual growth rings. Recent evidence suggests that these rings can also provide a permanent record of exposure to some elements that fish are exposed to in industrial effluents. Microchemical analysis of the growth rings is typically accomplished by laser ablation inductively coupled mass spectrometry (LA-ICP-MS). Until now, divalent cations which can substitute for calcium during the assembly of the otolith structure (eg. Zn, Mn, Mg, and Sr,) have been studied. More recently, we have used LA-ICP-MS to analyze elements incorporated in the mineral and protein matrix of the otolith, including Se, which behaves as an anion in the environment and in biological tissues. LA-ICP-MS was used to analyze selenium in otolith annual growth rings from rainbow trout and brook trout that were captured from reference streams and from streams with elevated selenium downstream from coal mining activity. Results illustrate important differences in exposure patterns between the two species.

Application of image analysis for age determination in fishes: advances, benefits, limitations. D. Fitzgerald[1], M. Jones[2] and J. Tetreault[1] (PL)

[1]Ecometrix Incorporated, Brampton, ON; [2]Cape Breton University, Sydney, NS

Image analysis refers to the process of capturing images (still or video) and then using these images to collect data observations and complete interpretations. Such methods are routinely used in the service, manufacturing, and health care sectors, and in many instances, these uses have been automated with dedicated computer work stations. Comparable advances have occurred with the methods used to determine age in fishes. In the last two decades, computer-based image analysis of calcified structures like scales, opercula, and otoliths from fish has become the standard for age determination in marine

fishes. However, comparatively fewer situations exist where freshwater or diadromous fish are assessed for age with these methods. This expansion in use can be attributed to the reduction in the cost of the equipment required and the need to create an archive of information on fish populations. Key limits of the use of image analysis for age determination include the need for the validation of image-derived age, and training in the methods. Case studies will be used to review these and other related themes.

Analysis of age using different methods for five species of minnows from streams across Ontario: implications for assessment and management. J. Tetreault[1], D. Fitzgerald[1] and N. Ali[2] (PL)

[1]EcoMetrix Incorporated, Brampton, ON; [2]Environment Canada, Downsview, ON

Analyses were completed on five species of minnow sampled from different streams in Ontario to resolve the age structure evident in the populations and to identify the most efficient and accurate method for age determination. The minnow species studied were blacknose shiner *(Notropis heterolepis)*, creek chub *(Semotilus atromaculatus)*, lake chub *(Couesius plumbeus)*, northern redbelly dace *(Phoxinus eos)*, and pearl dace *(Margariscus margarita)*. Several of the species studied have been used for Environmental Effects Monitoring (EEM). Age determinations were completed for the minnow species with different bones that included the cleithra, lapillus otoliths, opercula, and scales. Age estimates among the cleithrium, otoliths, and operculum of sexually immature fish, usually less than about age III+ years, were generally consistent. By contrast, differences in age estimates among these bones became large when sexually mature fish were assessed. Estimates of maximum age were primarily derived from the interpretation of lapillus otoliths. These analyses identified that a diverse age distribution is evident in the minnow species considered. These analyses indicated scale interpretations produced the most variable estimates of age while the opercula and otolith were most consistent. The consequences of these age distributions on the assessment and management of data collected for the EEM fish surveys will be considered.

Asymmetries in skeletal elements of mummichogs (<u>Fundulus heteroclitus</u>) as indicators of environmental stress. M. M. Jones[1], S. Deveaux[2], S. Dove[1] and J. Tomie[1] (PL)

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Abstract

Estuarine ecosystems in Industrial Cape Breton have experienced varying degrees of anthropogenic influence over the past century. For this study, we chose three estuaries that reflect low, medium and high degrees of habitat degradation from human activities (i.e. Mira River, Sydney River, and the Sydney Tar Ponds, respectively). Mira River is a lightly impacted estuary with cottages, year-round residences, some aquaculture and a

commercial eel fishery. It often experiences closures to shellfish harvesting during warm summer months. Sydney River is a much more urbanized estuary, with light industrial influence and municipal sewage. The Tar Ponds estuary is highly urbanized, and until the 1990s, was the receiving estuary for >100 years of unregulated industrial activities from former steel operations. The Tar Ponds are the repository of more than 700,000 tonnes of toxic waste containing polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), heavy metals, and until 2005, municipal sewage. This study investigated the use of calcified skeletal elements of a ubiquitous, small estuarine fish, the mummichog *(Fundulus heteroclitus)* as bioindicators of ecosystem health. Mummichogs show high site fidelity and are thus often used as sentinel species for Environmental Effects Monitoring programs in the Atlantic Provinces. We examined differences in developmental stress using fluctuating asymmetry (FA), with the assumption that fish developing in stressful environments exhibit greater degrees of bilateral asymmetry or lopsidedness. Right versus left differences in pectoral fin lengths, pelvic fin lengths, snout lengths, scale counts along the lateral line, and fin ray counts on pectoral and pelvic fins were analyzed for asymmetries. In addition, mummichogs cleared and stained with Alizarin Red-S were examined for bone deformities and abnormalities in their fins. Mummichogs from the Tar Ponds showed higher incidences of FA and deformities than mummichogs from Sydney River and Mira River. The potential utility of using FA and deformities in skeletal elements as bioindicators will be discussed.

Introduction

Industrial Cape Breton (i.e. Cape Breton Regional Municipality, including the city of Sydney) provides an excellent opportunity to study the effects of prolonged industrial and municipal activities on aquatic organisms in estuaries ranging from relatively clean to one of the most toxic in Canada, the Sydney Tar Ponds (Furimsky 2002). Our research project is particularly timely, given that in 2004, the governments of Canada and Nova Scotia agreed to fund and facilitate the remediation of the Sydney Tar Ponds and Coke Ovens sites (AMEC 2005). Various components of the large scale clean-up project have already begun, and work in the Tar Ponds has been scheduled for spring 2008 (W. Kaiser, Sydney Tar Ponds Agency, pers. comm.).

Muggah Creek (which includes the South and North Tar Ponds) is a shallow tidal estuary and is the repository of more than 700,000 tonnes of contaminated sediments containing polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), heavy metals (e.g. arsenic, cadmium, chromium, iron, lead, and zinc), and until 2005, municipal sewage (Matheson *et al.* 1983; Furimsky 2002; AMEC 2005). Concern surrounding the biological impact of contaminants from the Tar Ponds surfaced in the 1980s after government studies measured high levels of contaminants in soils in the Tar Ponds, and tissue samples of lobster and mussels in the Sydney Harbour (Matheson *et al.* 1983). To date, although many reports have been written about contaminant assessments (air, water and soil) in this watershed (Vandermeulen 1989; Acres Intl. Ltd. 1991; JWEL-IT Joint Venture 1996; AMEC 2005) very few have dealt with aquatic organisms, and fewer still

with fishes (Vignier *et al.* 1994; Vandermeulen and Mossman 1996; Foulds 2001; Jones 2007). Although there are existing biological studies of remediated systems elsewhere in North America such as New Bedford Harbour, Massachusetts, Elizabeth River, Virginia, and Hudson River, New York, (e.g. Weis and Weis 1989; Van Veld *et al.* 1991; Nacci *et al.* 1999; Monosson *et al.* 2003; McMillan *et al.* 2006), the Tar Ponds and adjacent areas pose some unique problems given their sheer size, the duration and extent of contamination, and proximity to residential areas. During the remediation process, Environmental Effects Monitoring (EEM) is required to regulate and mitigate numerous potential effects of contamination on fish habitat (AMEC 2004).

EEM is an approach used to assess the health of fish and fish habitat as a result of anthropogenic discharge (Environment Canada (EC) 2003). The EEM protocol compares several aspects of an impacted aquatic ecosystem with reference sites, including: 1) adult fish population surveys (e.g. length, weight, and body somatic indices), 2) benthic invertebrate surveys, 3) ecotoxicology analyses (e.g. metals for mining sites (EC 2002), and dioxins for pulp and paper mill sites (EC 1997, 2003)) of edible fish tissues, and 4) monitoring the aquatic environment for varying levels of effluent discharge (EC 2003). In addition to the factors analyzed by EEM, other characteristics may also prove useful in assessing fish health, such as the degree of Fluctuating Asymmetry (FA)(Møller and Swaddle 1997, Palmer and Strobeck 1997, Erisken *et al.* 2008) and the frequency of phenodeviance (Bodammer 1993, Møller and Swaddle 1997, Eriksen *et al.* 2008). FA and phenodeviance are measures of developmental stability, which refers to the competence of an organism to construct a planned phenotype under a given range of environmental and genetic conditions (Møller and Swaddle 1997). Higher incidences of FA and phenodeviances should correlate with higher degrees of developmental instability. FAs are diminutive, accidental deviations from symmetry in otherwise bilaterally symmetrical characters, whereas phenodeviances are relatively large and conspicuous morphological anomalies such as extra or missing bones (Van Valen 1962; Bodammer 1993; Møller and Swaddle 1997). FA and phenodeviance have recently attracted considerable interest because they appear to represent relatively simple methods to identify sublethal stress exposure.

The main objective of this study is to examine the potential use of FA in conjunction with several traditional EEM measures (i.e. condition factor, liver somatic index (LSI) and gonad somatic index (GSI)) to track the effectiveness of Tar Ponds remediation. The mummichog, *Fundulus heteroclitus* (Linnaeus 1766), was selected as the focus species for this research because it is an abundant, resident forage fish that is widely distributed in shallow, brackish coastal waters from the Gulf of St. Lawrence to northeastern Florida (Scott and Crossman 1973). In addition, the mummichog has been used for decades in environmental, toxicological, physiological, embryological and genetic studies and its responsiveness to contaminant exposure has been well documented (Weis and Weis 1989; Van Veld *et al.* 1991; Kirchhoff *et al.* 1999; Nacci *et al.* 1999; Dubé *et al.* 2002 and references therein; Monosson *et al.* 2003; McMillan *et al.* 2006).

Methods

Study sites

As part of a larger research project, this study focused on condition analyses of mummichogs collected from the heavily contaminated Sydney Tar Ponds (46°08'N, 60°12'W) and two reference sites in Industrial Cape Breton: Sydney River (46°06'N, 60°14'W) and Mira River (46°03'N, 60°01'W). The South and North Tar Ponds comprise the brackish and marine portions of the Muggah Creek Estuary, which empties into the South Arm of Sydney Harbour. Mira River is a lightly impacted estuary with cottages, year-round residences, recreational boating, and eel and oyster fisheries. It sometimes experiences closures to shellfish harvesting during warm summer months. Sydney River is a much more urbanized estuary, with light industrial influence and municipal sewage. The three sampling localities were similar in temperature, dissolved oxygen (DO) and salinity ranges throughout June to August 2006 (Table 1), with the exceptions of a slightly higher DO range in Mira River and a narrower salinity range in Sydney River.

Study species

Mummichogs were selected as the sentinel species due to their suitability as biomonitors: they show high site fidelity and small home range areas, they come into contact with the contaminated sediments through feeding and other behaviours, and they are ubiquitous and abundant throughout estuarine environments in eastern North America.

Table 1. Range of environmental parameters for each study site during sampling periods in June to August 2006.

Study Site	Water Temperature (°C)	Dissolved Oxygen ($mg \cdot L^{-1}$)	Salinity (parts per thousand)
Mira River	17.8 – 20.8	6.3 – 7.8	4.5 – 18.2
Sydney River	16.9 – 21.2	5.7 – 6.2	10.6 – 22.0
Tar Ponds	17.5 – 20.9	3.8 – 6.2	1.2 – 19.8

Indeed, in Industrial Cape Breton, Jones (2007) found that the mummichog was the dominant species in local estuaries and represented 89.8% of the total catch in minnow traps overall. The mummichog was dominant at all sites, and consistently comprised a significant portion of the catch in minnow traps, from a low of 65.5% of the catch at Sydney River, to a high of 95.4% of the catch at Mira River. The mummichog comprised 94.3% of the fish caught in minnow traps in the Tar Ponds.

For this study, mummichogs were collected using minnow traps baited with white bread on July 22, 2006 at Mira River, July 25th and 26th in the Tar Ponds, and July 29th at Sydney River. Mummichogs were anaesthetized using clove oil (in accordance with CBU Animal Care Committee guidelines) and preserved in 10% formalin. For this project, a total of 80 mummichogs were processed (9 females and 11 males from Mira River, 18

females and 19 males from Sydney River, and 13 females and 10 males from the Tar Ponds).

Prior to processing, each fish was digitally photographed (left and right lateral views) for archival purposes. All fish were weighed using a digital balance (±0.001g) and then measured (±0.01mm) using Mitutoyo® digital calipers linked to a desktop computer. The following measurements were recorded: total length (TL; tip of snout to end of caudal fin), standard length (SL; tip of snout to end of the vertebral column or hypural plate), and left and right (L-R) snout lengths (anterior tip of snout to the margin of the orbit), and L-R pectoral and pelvic fin lengths. L-R measurements in eye diameters were also recorded but the results will not be discussed here. To reduce experimenter bias on symmetry measurements, all right side measurements of each fish were recorded first, followed by all left side measurements. In addition, two complete sets of L-R measurements were taken at different sittings.

After the measurements were completed, the fish were dissected to obtain liver and gonad weights. All other internal organs were also removed from the fish in preparation for clearing and staining. The mummichogs were cleared and stained using a method simplified from Hangar (1969), which consisted of: 24 hours in 3% hydrogen peroxide (H_2O_2); 24 hours in 1% potassium hydroxide (KOH); 24 hours in 1% KOH with alizarin red-S; and then the specimens were placed in glycerol for storage until fin rays could be counted. Additional post-stain counts of other calcified structures were also recorded, including caudal fin rays, dorsal fin rays, L-R scale counts along the lateral line, and L-R branchiostegal counts; however, these additional counts are not presented here. Pectoral and pelvic fins were clipped from the fish, viewed under a dissecting microscope for counts of the number of branched rays, and digitally photographed for archival purposes. L-R pectoral and pelvic fin ray counts were done by two observers and were rechecked if there was a discrepancy between observers.

Data analysis

Fulton's condition factor (K) is commonly used to assess the condition of fish by assuming that the plumpness of an individual is indicative of health (Moyle and Cech 2004). K is found by comparing the weight (in grams) and total length (in centimeters) of each fish ($K = W/L^3$).

For all L-R measurements (snout, pectoral fin and pelvic fin lengths), the two measurements for each parameter were averaged. The right value was then subtracted from the left value and converted to an absolute difference. The absolute difference was then divided by the standard length of the fish to standardize the measurements by fish size prior to analysis. Standardization was necessary because studies have shown that asymmetry may be correlated to condition factor (Eriksen *et al.* 2008).

Liver somatic index (LSI) and gonad somatic index (GSI) are both used as indicators of aquatic health. The LSI is calculated by dividing the liver weight of a specimen by the total body weight of the fish, then multiplying by 100. The GSI is calculated by dividing the gonad weight by the total body weight of the fish, then multiplying by 100. To

account for differences in reproductive allocation as a result of gender and the presence of eggs, mummichogs were broken down into three categories: males, females without eggs, and females with eggs. The expected pattern in this study was for the Tar Ponds mummichogs to have increased liver weights (due to increased detoxification activity) and decreased gonad weights (due to decreased reproductive allocation) relative to Sydney River and Mira River, in accordance with nationally summarized EEM results (EC 2003).

All data were plotted and analyzed using SigmaPlot 9.0 and SigmaStat 3.1. One-way ANOVAs followed by Dunn's multiple comparison tests were run on all data. When assumptions of normality and equal variance could not be met, Kruskal-Wallis one-way ANOVAs on ranks followed by Holm-Sidak multiple comparison tests were run instead.

Results and Discussion

The mummichog samples from each site differed in their weights and lengths, and consequently their condition factors (Table 2, Fig. 1a). Overall, Mira River mummichogs were the largest, followed by Sydney River, then the Tar Ponds. Mira River mummichogs had significantly higher condition factors than the other two sites (Fig. 1a; Kruskal-Wallis (K-W) One-way ANOVA on ranks: $H = 31.9$, $df = 2$, $p < 0.001$); however, Sydney River was not significantly different from the Tar Ponds. Given the degree of contamination in the Tar Ponds (AMEC 2005), it was not surprising that Tar Ponds fish had the lowest condition factors. On the other hand, national EEM results showed that the predominant response pattern for pulp and paper mill effluent studies was an increase in condition factor due to a nutrient enrichment effect (EC 2003). Despite decades of abundant nutrient enrichment from raw sewage flowing into the Tar Ponds, there is likely a greater detrimental effect on fish condition due to the high levels of contaminants (PCBs, PAHs and heavy metals). A study to assess the current level of contamination in biota and sediments from the Tar Ponds is currently underway (L. Rockwell, R. Russell and M. Jones, unpublished).

Table 2. Average weights, total lengths and standard lengths (± standard deviation) of mummichogs from Mira River, Sydney River, and the Tar Ponds. Sample sizes are in parentheses.

Study Site	Weight [g]	Total Length [mm]	Standard Length [mm]
Mira River	3.9 ± 1.5 (20)	63.8 ± 6.5 (20)	53.7 ± 6.0 (20)
Sydney River	3.0 ± 1.3 (36)	60.9 ± 7.0 (37)	51.1 ± 6.5 (37)
Tar Ponds	1.7 ± 1.1 (23)	52.3 ± 8.8 (21)	44.6 ±7.2 (23)

There were interesting results with LSI and GSI that warrant further investigation. Firstly, LSI results (Fig. 1b) did not follow the same pattern as condition factor. Tar Ponds mummichogs had the highest LSI, followed by Mira River, then Sydney River. Secondly, there was much variation in LSI, which is shown by the large standard error bars (Fig. 1b) and the statistical results from a Kruskal-Wallis ANOVA on ranks. There was a significant difference in LSI between Mira River and Sydney River, but not between Sydney River and the Tar Ponds, which suggests that many of the Sydney River mummichogs often ranked higher than Tar Ponds mummichogs, despite the lower site average in LSI for Sydney River. For GSI, Tar Ponds mummichogs had the highest average for each category of mummichogs (males, females without eggs and females with eggs). Small sample sizes and high variability within each category across sites precluded statistical analysis for the GSI data. National EEM studies found that the predominant response patterns seen in fish were an increase in LSI and a decrease in GSI at sites impacted by pulp and paper mill effluent (EC 2003). These responses are believed to be indicative of some form of metabolic disruption or impairment of endocrine functioning in combination with a nutrient enrichment effect. Some individual EEM studies report different findings than the national summary. For example, Leblanc *et al.* (1997) found a marked increase in reproductive investment with higher fecundity and GSI in fish populations living immediately downstream from bleached kraft mill effluent (BKME). Various hypotheses have been offered to explain the observed increased investment in reproduction from exposure to industrial effluents, including nutrient enrichment, estrogenic effects, or genetic adaptation of a population under chronic stress (LeBlanc *et al.* 1997; Kirchhoff *et al.* 1999, references therein). All of these hypotheses could be explored for mummichogs in the Tar Ponds.

The absolute L-R differences in body measurements (snout, pectoral fin, and pelvic fin lengths) showed high degrees of variability within each site (Fig. 2a,b). The only measurement that showed a significant difference among sites was snout length (K-W One-way ANOVA on ranks; $H = 7.51$, $df = 2$, $p = 0.023$). The Tar Ponds mummichogs exhibited higher degrees of fluctuating asymmetry in snout length compared to Sydney River. For pectoral fin lengths, the Tar Ponds mummichogs showed the highest degrees of asymmetry, followed by Sydney River and then Mira River (Fig. 2b); however, this trend was not significant (K-W ANOVA on ranks; $H = 2.904$, $df = 2$, $p = 0.234$), perhaps due to low sample sizes. Another factor that may have affected the high level of variability is experimenter error. Caliper measurements on small body parts are challenging, and slight shifts in caliper angles may result in large measurement errors, although great care was taken in this study to ensure the accuracy and repeatability of caliper measurements.

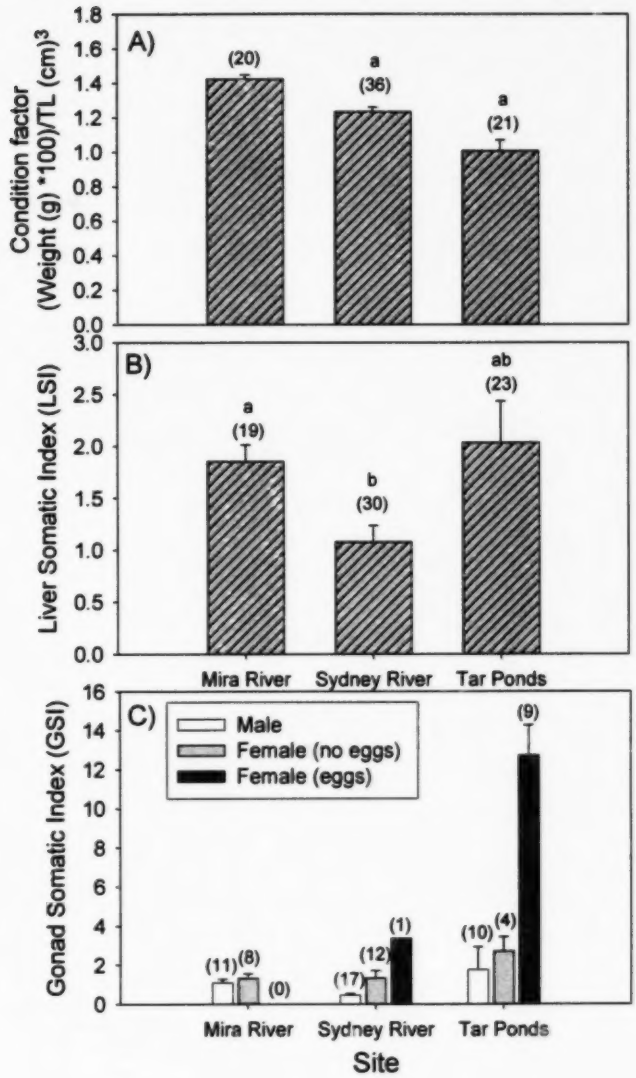

Figure 1. Average site differences in mummichog A) condition factor, B) LSI C) (GSI). Bars that do not share the same letter in A) and B) are significantly different (Kruskal-Wallis One-way ANOVA on ranks: A) $H = 31.9$, $df = 2$, $p < 0.001$; and B) $H = 12.54$, $df = 2$, $p = 0.002$). Sample sizes in parentheses; error bars = one standard error of the mean.

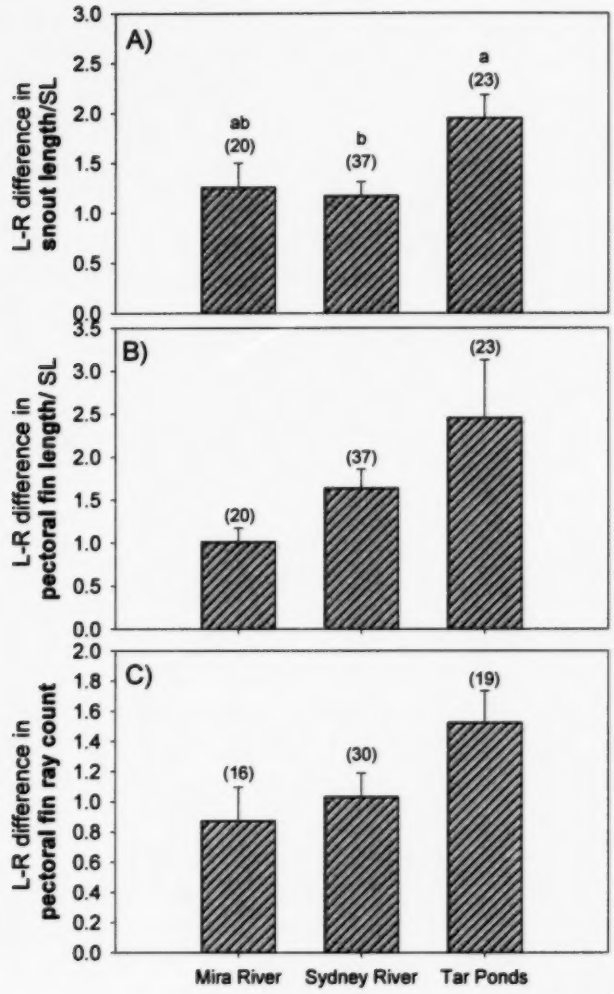

Figure 2. Average absolute left-right asymmetries in A) snout length, B) pectoral fin length, and C) pectoral fin ray counts. Body size effects on left-right differences in snout length and pectoral fin length were standardized by dividing by the standard length (in mm) of each sample before statistical analyses. Bars that do not share the same letter in A) are statistically significantly different (Kruskal-Wallis ANOVA on ranks; $H = 7.51$, $df = 2$, $p = 0.023$). Sample sizes are in parentheses; error bars represent one standard error of the mean.

Additional morphometric (i.e. body measurements) studies are currently underway to compare caliper measurements with digital image analyses.

To examine asymmetry in fish meristics (body parts that can be counted), absolute L-R differences in pectoral and pelvic fin ray counts were compared among sites. There was no

significant difference among sites for the absolute L-R difference in pelvic fin ray counts (K-W ANOVA on ranks; H = 1.064, df = 2, p = 0.587). For pectoral fin ray counts, Tar Ponds mummichogs showed the highest degree of asymmetry, followed by Sydney River, then Mira River (Fig. 2c). However, here again, these results were not statistically significant (K-W ANOVA on ranks; H = 4.64, df = 2, p = 0.098), perhaps due to small sample sizes. A closer examination of the degree of asymmetries in fin ray counts revealed additional differences among sites (Table 3). One surprising finding was the prevalence of asymmetries in all populations. For example, 56.3% of the Mira River mummichogs sampled exhibited asymmetries in their pectoral fin ray counts (Table 3). No mummichogs from the Tar Ponds had an equal number of pectoral fin rays on either side, the majority (68.4%) exhibited a difference of one fin ray on either side, but some (5.3%) showed a difference as large as four fin rays on either side. The prevalence of asymmetries in pelvic fin ray counts was not as great (Table 3), perhaps because the pelvic fins are more constrained than pectoral fins in the possible number of rays (4 to 8 branched rays for pelvic fins, as opposed to 10 to 17 branched rays for pectoral fins in the present study; Scott and Crossman (1973) report 6 or 7 principal pelvic fin rays as opposed to 16 to 21 principal pectoral rays.

It is worth noting that for many fins from Tar Ponds mummichogs, the number of branched rays were problematic to count (Fig. 3). Mummichogs from Mira River and Sydney River typically had fins with regular branching patterns; whereas Tar Ponds specimens had fin rays that: 1) often did not branch where it was apparent they should, 2) were missing altogether, 3) look like two fused together, or 4) were gnarled or twisted. Indeed, 9% of pelvic fins and 13% of pectoral fins from Tar Ponds mummichogs were completely uninterpretable. Only 1% of Sydney River pelvic fins were uninterpretable, and 0% of Mira River fins were uninterpretable.

Table 3. Degree of fin ray count asymmetries in mummichogs from Mira River (MR), Sydney River (SR) and the Tar Ponds (TP). The numbers indicate the percentage (%) of individuals that exhibited left-right differences in fin ray counts.

Left-Right	Pectoral Fins			Pelvic Fins		
Difference	MR	SR	TP	MR	SR	TP
0 rays	43.8	26.7	0	75.1	72.4	60
1 ray	25	50	68.4	25	27.6	33.3
2 rays	31.3	16.7	15.8	0	0	6.7
3 rays	0	6.7	10.5	--	--	--
4 rays	0	0	5.3	--	--	--
Total % Asymmetrical	56.3	73.4	100	26	27.6	40

Summary statements

Overall, there appear to be promising results for the potential utility of FA as a bioindicator in Tar Ponds mummichogs. Flaws in symmetry may reflect perturbations experienced during ontogeny and thereby represent responsive, consistent indices of preceding environmental challenges (Møller and Swaddle 1997). However, it is important to note that the hypothesized relationship between sublethal stress and FA has not always been supported (Palmer 1996; Cuervo and Restrepo 2007). For the present study, the traits with the greatest potential, which therefore warrant further investigation, include L-R differences in snout length, pectoral length and pectoral fin ray counts. In conjunction with this, a logical follow-up study would be an investigation of the incidence of malformations or phenodeviance (as evidenced in Fig. 3) as bioindicators in Tar Ponds mummichogs. The traditional EEM measures employed in this study (condition factor, LSI and GSI) resulted in some ambiguous results that also warrant further investigation. It was obvious throughout this study that Tar Ponds fish do exhibit signs of compromised health, and the challenge will be to capture the most meaningful elements for baseline information. The Tar Ponds and other sites that are contaminated with such a complex mixture of pollutants may require both traditional and innovative approaches to assess the effectiveness of environmental remediation.

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Comparative analysis of age of pearl dace (<u>Margariscus margarita</u>) used for environmental monitoring of headwater systems in Ontario. ***D. Parks[1] and D. Fitzgerald[2] (PL)***

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It has been acknowledged that small-bodied fish can represent an integral component of an Environmental Effects Monitoring (EEM) program for mining sites located in the headwaters of watersheds that lack large-bodied fish. Pearl dace *(Margariscus margarita)* were used to meet the biological monitoring requirements as directed by Environment Canada under the Metal Mining Effluent Regulations, for an ore extraction mine in northern Ontario. The age analysis of the pearl dace involved different interpretations of the otolith, a bone located in the head and the preferred aging structure in most freshwater fish species. The purpose of this presentation is to contrast the differences in estimated ages of otolith interpretation from the traditional "cut and burn" compared with the image analysis of polished otoliths. These results show the traditional cut and burn method tends to underestimate the age of older fish, while the image analysis method allows for accurate and precise age estimate of older fish. Age estimates of younger fish were similar for both methods. Identification of older pearl dace indicates a longer generation time and lower productivity and suggests that such forage fish populations may be vulnerable to over-exploitation and compromise long-term monitoring efforts and objectives. Implications of incorrect age determination on the biological interpretation of this EEM study will also be discussed in the context of northern headwater systems.

Age of finescale dace from streams in northern Ontario. D. Fitzgerald[1], J. Tetreault[1] and N. Ali[2] (PO)

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Assessment of age of finescale dace *(Phoxinus neogaeus)* was completed for fish sampled from streams in northern Ontario. These analyses are consistent with the requirement to assess resident fish populations near metal mines for the Environmental Effects Monitoring (EEM) program. The age of finescale dace was estimated with the lapillus otolith, opercula, and cleithra to identify the age of the fish and to resolve the most accurate and efficient bone(s) to use for this purpose. These analyses identified the otolith yielded the highest age estimates followed by the opercula and cleithra. Estimates of age from the different bones will be presented. The maximum age estimated for finescale dace was >10 years and this was higher than previous studies for this species. However, such maximum ages are consistent with other minnow species previously assessed for age with otolith analyses, such as creek chub *(Semotilus atromaculatus)*, lake chub *(Couesius plumbeus)*, and fallfish *(S. corporalis)*. These observations indicate the otolith or the operculum should be used to age finescale dace. It is possible to infer the short growing season and low productivity of streams in northern Ontario may explain why finescale dace can commonly live >10 years. Thus, accurate age estimates for minnows is required in order to fully represent the natural variability in attributes of resident fish populations in streams for the EEM program.

Effects-based Pesticides Research (Poster Session)

Session Chair/ Président Vince Palace

Site fidelity of golden shiners (Notemegonus chrysoleucas) and their use in effects-based assessments of pesticides by DFO's Center for Environmental Research on Pesticides (CERP). V. Palace[1], A. Goodmanson[2], J. Struger[3], S. Mittermuller[1], J. Hare[2], K. Wautier[1], C. Baron and L. Peters[2] (PO)

[1]Fisheries and Oceans Canada, Winnipeg, MB; [2]University of Manitoba, Winnipeg, MB; [3]Environment Canada, Burlington, ON

The Canadian Department of Fisheries and Oceans (DFO), Center for Environmental Research on Pesticides (CERP), has adopted the use of small-bodied fish in an Environmental Effects Monitoring (EEM) framework for assessing the potential effects of pesticide use in southern Ontario. Specifically, golden shiners *(Notemegonus chrysoleucas)* have been captured and assessed from several locations of Twenty Mile Creek, an intermittent flow creek in southern Ontario that often has multiple pesticide inputs from adjacent vineyards, orchards, a recreational golf course and urban runoff. While results suggest that pesticides may be affecting gonadal steroidogenic capacity and recruitment, uncertainty regarding mobility of the fish between study sites precludes the ability to link these effects with exposures to pesticides at each specific site.

To address this knowledge gap, resident golden shiners at six sites on Twenty Mile Creek were marked using site-specific colours of visible implant elastomer (VIE). Recapture records at three periods throughout the summer are presented as a measure of site fidelity and pesticide exposure regimes.

Biological effects of water-borne exposures of larval American lobster to pesticides and 4-nonylphenol. W. Fairchild[1], K. Doe[2], J. Arsenault[1], K. Benhalima[1], A. Cook[2], P. Jackman[2], S. Reebs[3] and M. Comeau[1] (PO)

[1]Fisheries and Oceans Canada, Moncton, NB; [2]Environment Canada, Moncton, NB; [3]Université de Moncton, NB

This study observed the effects of low-level water-borne exposures of 4-nonylphenol (NP) and pesticides on survival, growth, molting success, histology and behavior of lobster larvae. In the 12-week chronic exposures, survival of stage IV lobster was affected by endosulfan, and growth and molting were delayed by atrazine and endosulfan. In the 4-week sub-chronic exposures, survival of stage III lobster was significantly affected by nonylphenol (100 $\mu g \cdot L^{-1}$) and endosulfan (1 $\mu g \cdot L^{-1}$), and development to stages IV and V was delayed by endosulfan at 0.1 $\mu g \cdot L^{-1}$. In acute lethality tests (48 h), endosulfan had significant effects on survival of stage I (LC50 = 2.51 $\mu g \cdot L^{-1}$ a.i.) and IV lobster (LC50 = 3.98 $\mu g \cdot L^{-1}$a.i.); and azinphosmethyl (LC50 = 3.16 $\mu g \cdot L^{-1}$a.i.) and chlorpyrifos (LC50 = 0.35 $\mu g \cdot L^{-1}$a.i.) had significant effects on survival of stage IV lobster. Histological observations showed alteration in hepatopancreas cells of lobster exposed to all the pesticides and NP. Those exposed to endosulfan showed the most extensive cellular abnormalities, but unlike the other pesticides, did not show extensive change of the cuticle layers. Locomotor and settling behavior of the lobster was not affected by atrazine and hexazinone. The locomotor activity was significantly affected by endosulfan (0.1 $\mu g \cdot L^{-1}$) and NP (10 $\mu g \cdot L^{-1}$).

Histological observations of American lobster (Homarus americanus) larvae exposed to pesticides and nonylphenol. K. Benhalima[1], M. Comeau[1], J. Arsenault[1], K. Doe[2], P. Jackman[2] and W. Fairchild (PO)

[1]Fisheries and Oceans Canada, Moncton, NB; [2]Environment Canada, Moncton, NB

Alterations in cell, tissue and cuticle structure of American lobster *(Homarus americanus)* larvae exposed to pesticides and nonylphenol in laboratory experiments were observed using histology. Larvae were exposed during their molt from stage III to IV (i.e., metamorphic molt with the first appearance of chelipeds) to various concentrations of currently used pesticides and nonylphenol including: endosulfan (insecticide), atrazine (herbicide), hexazinone (herbicide) and nonylphenol (pesticide formulant). The exposure ended seven days after their molt to stage IV, and animals that survived the experiments were preserved in Bouin's solution for histological observations. Compared to control animals, lobster larvae exposed to different contaminants showed no tissue damage in the eyes, intestines and gills; however, alterations in the cuticle layer and hepatopancreas cells were observed. All animals exposed to pesticides and nonylphenol showed

measurable alterations of the hepatopancreas cells, in particular, and those exposed to endosulfan showed the most extensive cellular abnormalities. Unlike the other pesticides and the nonylphenol that caused extensive changes to the cuticle layers, endosulfan did not seem to affect the exoskeleton. The various effects detected in this study could affect various physiological functions, such as the absorption and secretion by the hepatopancreas, growth determined by molting success, and the capacity of the exoskeleton to resist disease.

Current use pesticides in British Columbia salmon habitat. K. Harris[1], K. Tierney[2], N. Dangerfield[1], M. Woudneh[3], T. Brown[1], C. Kennedy[2] and P. Ross[1] (PO)

[1]Fisheries and Oceans Canada, Sidney, BC; [2]Simon Fraser University, Burnaby, BC; [3]Axys Analytical Services, Sidney, BC

The use of current-use pesticides (CUPs) represents a growing conservation concern to Fraser River salmon habitat. We carried out a study of CUPs in two salmon-bearing tributaries of the Fraser River and one reference site on British Columbia's central coast, comprising urban (Musqueam Creek), agricultural (Nathan Creek), and remote (Koeye River) areas. Water, sediment, air, and coho salmon (*Oncorhynchus kisutch*) smolt samples were collected from each site. Total pesticide concentrations in three of the four matrices were highest at the agricultural site (water: 162 ng L^{-1}, sediment: 1475 ng L^{-1}, air: 1285 ng L^{-1}), while concentrations detected in biota were highest at the urban site (54.5 ng L^{-1}). Correlations between log K_{ow} and observed water:sediment ratios (agricultural $r^2 = 0.36$, urban $r^2 = 0.64$) and between Henry's Law coefficients and air:water ratios (agricultural $r^2 = 0.55$, urban $r^2 = 0.09$) revealed the importance of physicochemical properties in determining the fate of different pesticides in aquatic environments. Inter-site differences likely reflect the heavy agricultural use of CUPs near Nathan Creek, a legacy of organochlorine contamination in Musqueam Creek, and atmospheric delivery of pesticides to the Koeye River. Differences in concentrations and patterns of CUPs in different environmental compartments underscore the complex nature of defining 'exposure' and ultimately of assessing risk of adverse health effects in sensitive salmonids.

Oocyte atresia in stickleback sampled in a marsh receiving agricultural runoff at Isle Verte in the St. Lawrence Estuary. C. M. Couillard[1], R. L. Roy, M. Lebeuf, C. Deblois[2] and M. Mercure[3] (PO)

[1]Fisheries and Oceans Canada, Mont-Joli, QC; [2]Centre d'expertise en analyse environnmentale du Québec, Ste-Foy, QC; [3]Université du Québec à Montréal, Montreal, QC

Coastal marshes of the St. Lawrence Estuary are critical habitats for several fish species. They are contaminated by pesticides from urban or agricultural runoffs with possible impacts on fish reproduction. Gonad histopathological biomarkers (intersex in males and oocyte atresia in females) were evaluated in threespine stickleback *(Gasterosteus aculeatus)* collected in 2003 in four coastal marshes. Two marshes receive agricultural runoffs (Isle Verte (IV), Trois-Pistoles (TP)), one receives urban runoffs (Pointe-au-Père (PP)) while Bic was considered as the reference site. No intersex condition was found, indicating that sticklebacks were not exposed to toxic concentrations of estrogenic chemicals at critical times of their development. However, an increase in the proportion of atretic oocytes with time was observed at the two agricultural sites

(IV and TP) and not at Bic and PP. In order to examine if this response was related to differences in fish size among sites or among sampling times, a larger number of fish was sampled at IV and PP in 2006. Large-size fish (>69 mm) begun spawning earlier at IV compared to PP. At the end of the spawning season, a reduction in the proportion of large-size fish captured was observed at IV. At the same time, a higher proportion of atretic oocytes was observed in these large-size fish compared to small-size fish. Several factors could have contributed to this response, including elevated concentrations of pesticides (atrazine and metholachlor) and nitrates in a freshwater tributary and severe oxygen depletion in tidal pools. This study demonstrates the importance of considering the effect of fish size and of multiple environmental factors in spatiotemporal comparisons of prevalence of oocyte atresia in stickleback.

Effects of pesticide exposure in freshwater on subsequent sea water growth of Atlantic salmon smolts. L.E. Burridge[1], W. Fairchild, J. Arsenault[1], A. Cook[2], D. MacLatchy[3] and K. Haya[1] (PO)

[1]Fisheries and Oceans Canada, St. Andrews, NB; [2]Environment Canada, Moncton, NB; [3]Wilfrid Laurier University, Waterloo, ON

Some compounds reduce subsequent growth of a proportion of Atlantic salmon when the smolts are exposed in freshwater during late parr-smolt transformation (PST) then transferred to seawater. Field sampling for pesticide residues in freshwater and laboratory experiments have been conducted to determine if pesticides commonly used on Canada's east coast result in reduced growth of salmon. In 2004 and 2006, water samples were taken from rivers across NB and NS with an emphasis on agricultural watersheds. Hexazinone was one of the pesticides found in coastal rivers. In 2004, Atlantic salmon smolts were exposed to pulsed doses of the herbicides atrazine and hexazinone. In 2006, fish were exposed to the pulsed doses of the insecticide chlorpyrifos (nominal concentration = 0.06, 0.6 or 3.0 μg L-1) or the fungicide chlorothalonil (nominal concentration = 2, 75 or 150 μg L-1). The fish were held for up to six months to assess growth. Exposure to two 24 h pulses of atrazine at 20 μg L-1 resulted in a higher proportion of small smolts in July than in the control group. More large smolts were observed in fish treated with hexazinone. Specific growth rates (SGR) of smolts exposed to chlorothalonil or chlorpyrifos were no different from rates observed in control fish except in one case, where fish exposed to chlorothalonil at 75 μg L-1 had higher SGR from July to November than control fish. In addition to no effect of chlorpyrifos on growth, there was no effect of exposure on brain acetylcholinesterase activity.

Can the hormone Mimic® Tebufenozide be safely used in forest spray programs in Newfoundland? J.F. Payne[1], L. Fancey[1] and J. Hanlon[2] (PO)

[1]Fisheries and Oceans Canada, St. John's, NL; [2]Oceans Ltd., St. John's, NL

Studies were carried out under the National Pesticide Research Fund (a) to provide guidance on the controversial issue of the size of buffer zones required to protect salmon and other freshwater fish from Mimic® (active ingredient, tebufenozide), a pesticide slated for use in forest spray programs and (b) to assess the potential of the chemical to act as a molting hormone disruptor in lobster in case of runoff into the nearshore marine environment. Acute toxicity tests (48-96hr LC50s) with juvenile salmon, trout and codfish as well as capelin larvae indicated that

tebufenozide has a relatively low toxicity potential – in the 2-10 $mg \cdot L^{-1}$ range. Although osmoregulatory fitness as assessed by seawater challenge tests was affected in Atlantic salmon upon chronic exposure (~0.2 $mg \cdot L^{-1}$ in 10 static 8h pulses over a 2 month period), fitness was not affected in salmon or trout exposed to relatively high levels of the pesticide in water (~ 2 $mg \cdot L^{-1}$) for 48hrs. Similarly, although histopathological effects were observed in lobster chronically exposed to Mimic® (~1.5 $mg \cdot L^{-1}$ in 20 static pulses over a five month period), there were no effects on animal molting, premature egg laying or feeding. Overall, the results suggest that there is little need for buffer zones in association with forest spray programs where any deposits are expected to only occur transiently and in the low $\mu g \cdot L^{-1}$ range. Should extra assurance be required, buffer zones in the 10-25m range seem adequate.

Effects of pesticides on steroid production by gonadal tissues from the three-spined stickleback (Gasterosteus aculeatus), a small sentinel fish species of the St. Lawrence Estuary. C. Le Mer[1], R. Roy and D. Maltais (PO)

[1]Fisheries and Oceans Canada, Mont-Joli, QC

The adaptation ability and breeding cycle of the three-spined stickleback, *Gasterosteus aculeatus,* have been extensively studied over the past decades. Recent studies showed that this teleost fish species is a good biological model to study endocrine disturbances. 11-ketotestosterone (11-KT) is the primary androgen in a large majority of male teleosts, including the stickleback, while 17β-estradiol (E2) is the main sex hormone found in females. The aim of this study was (I) to assess levels of steroid hormones (11-KT and E2) produced by gonads from mature male and female sticklebacks sampled at Isles-Verte (an agricultural site) and Pointe-au-Pere (a non-agricultural control site), two locations of the Saint-Lawrence Estuary, during the spawning season, and (II) to determine the effects of pesticides on these sex steroid levels. To that end, the gonads were incubated for 24h in a culture medium and steroid quantification was carried out using ELISA kits. In males, 11-KT levels, as high as 1 ng/ml, were measured and showed variations that could be in relation with the stage of gonadal development. In contrast to males, the female sticklebacks presented elevated concentrations of E2. However, differences between the two sampling sites were observed. It was also established that the females were in better condition at Pointe-au-Pere, as determined by a higher LSI level than those sampled at Isles-Verte. Moreover, there were more fish with a smaller body size at the Isles-Verte site compared to Pointe-au-Pere site, indicating that the breeding is completed earlier at Isles-Verte. It has already been noticed that the proportion of atretic oocytes increased with time at Isles-Verte. These observations suggest possible reproductive dysfunction in female sticklebacks, which may be due to inputs of pesticides in the agricultural site.

Toxic effects of three pesticide formulations on the amphipod Corophium volutator. P. Walker[1] and J. Hellou[1,2] (PO)

[1]Dalhousie University, Halifax, NS; [2] Fisheries and Oceans Canada, Dartmouth, NS

Our study relates to examining the biological effects of three pesticide formulations that would be readily discharged in an estuary with fine grain sediments. The formulations are widely available and contain pesticides that have been used in Prince Edward Island (PEI), Canada

(White *et al.*, 2006). They were chosen from a series of pesticides classified according to toxicity criteria and to the volume used in PEI (Dunn, 2004). They are presently of interest from the perspective of government regulations.

The species used in our research, *Corophium volutator,* is an amphipod that lives in the mudflats of the upper Bay of Fundy. They are found scurrying around or buried just under the surface of the sediment during low-tide. *Corophium* were chosen for toxicity studies because they can be cultured in captivity, are widely distributed, taxonomically widespread, available in large numbers, have a short life cycle, and high reproductive potential. They are also quite sensitive to organic enrichment (Conlan, 1994). *Corophium* is located nearly at the bottom of the food chain; thus, results can be judged as representative of other potential chronic effects that could develop in higher marine life.

The commercial products Roundup, Lorsban and Bravo were studied. Glyphosate is the active ingredient in the Roundup formulation. It is the second most heavily used pesticide in the United States and has been described as "one of the most important agrochemical discoveries of the 20th century" (Caux, 1996). It is believed that the negative effects of the surfactant, polyoxyethylene tallow amine (POEA), greatly outweigh those of the glyphosate. Roundup has been shown to disrupt transcription which induces cell cycle dysfunction and is linked to the universal cell cycle, a primary cause of cancer.

Chlorpyrifos is present in Lorsban. It is a broad-spectrum insecticide which acts upon its targets by inhibiting the production of acetylcholinesterase. The subsequent accumulation of acetylcholine at neural junctions causes an over-stimulation of the peripheral nervous system. This leads to more energy being devoted to movement, and therefore less energy is available for other metabolic pathways such as reproduction (Roast, 1999). An increase in activity can also be associated with a decrease in lipid content.

Chlorothalonil is a broad-spectrum fungicide that is among the top five most heavily used fungicides in North America (Caux, 1996). It is considered very highly toxic to aquatic organisms. Chlorothalonil can delay reproduction, inhibit righting ability and diminish movement. Its main side-effect is to block glyceraldehyde 3-phosphate dehydrogenase, which is a required step in glycolysis, a biochemical pathway that is important in the formation of biologically essential molecules. Chlorothalonil is the active ingredient in the formulation Bravo.

The effects of these three pesticide formulations on the survival, behaviour, growth and lipid content of *Corophium volutator* were examined in two- and ten-day exposures to sediments spiked with pesticides at levels ranging from 0.1 to 1,000 $ng \cdot g^{-1}$ (dry weight). Tests were also performed to evaluate the survival of the animals relative to reduced salinity and increased levels of unionized ammonia. Conditions were set so that they would mimic those that could be expected in the field along with or instead of the discharge of pesticide after a rain event. Survival and weight, as a proxy for growth, fit under the population level effects. Behaviour, in terms of attraction or repulsion towards reference as opposed to spiked sediment, also fits under the population level effects. The latter sub-lethal cumulative effect was investigated as a potential tool to help predict and prevent further detrimental impacts. Lipid analysis was used to determine if a biochemical disturbance was taking place in exposed individuals.

A replacement of seawater with freshwater reaching a salinity of 9 ppt was associated with LC_{20}. This means that more than 80% of the water arriving in an estuary (32 ppt) inhabited by *C. volutator* would have to be replaced by freshwater in order to affect survival. According to available data on the levels of ammonia in sediment from PEI estuaries, the maximum levels that have been observed previously were 0.2 $mg \cdot L^{-1}$ (Phil Yeats, Personal Communication). Animals were tested up to a concentration of 3.5 $mg \cdot L^{-1}$ ammonia in seawater, but no effects were observed upon survival. This approach was based on the work of Alonso (2004), who used concentration of unionized ammonia from 0.02 to 1.19 $mg \cdot L^{-1}$ to test the behaviour of a freshwater snail.

Bravo, containing chlorothalonil (isopthalonitrile) did not affect survival in either short or long-term exposure, behaviour, or lipid as percent of total weight. However, when looking at the mean weight of lipid per animal, it is evident that a change took place. Between reference specimens and those exposed to Bravo, there was an increase in lipid weight of 100%. The weight of individual animals also increased (Table 1). This increase in lipid and animal weight is unrelated to concentration or duration of exposure. This indicates that more lipids are produced per animal exposed to Bravo, but that the percent of total weight is not altered. Kamrin (1997) reports chlorothalonil as toxic to marine organisms with a LD_{50} for trout, bluegill sunfish and water fleas of 49-70 $\mu g \cdot L^{-1}$. Amphipods survival was not affected by Bravo at concentrations ranging from 0.1 to 1,000 $ng \cdot g^{-1}$ (dry weight of sediment), with exposures for two or ten days.

The octanol-water partition coefficient (log K_{ow}) of Bravo is 3.1 as measured in our lab. This value can be used to estimate the relative distribution between water and sediment using the equilibrium partitioning theory. Under the laboratory set up, 100 times more pesticide will be present in the sediment than in water when comparing one gram to one millilitre, and therefore our higher exposure level is expected to be 1 $\mu g \cdot L^{-1}$. This lower cut-off for exposure levels can explain the observed optimum survival relative to the published data (Kamrin, 1997).

Chlorpyrifos, active ingredient in Lorsban, is a 'supertoxic' insecticide used extensively throughout North America. It causes over-stimulation of nerves, denying energy to other important biochemical processes. It is shown that Lorsban is in fact quite toxic to *C. volutator*. The reference animals did not have a very high survival rate during short term exposure (68%). At the highest concentration, 1,000 $ng \cdot g^{-1}$, every single animal in the tank died.

Trial 1 (Short Term)						
Cnctrn. of Bravo	Number of Amphipods	Weight of Amphipods	Mean Weight (mg)	Weight of Lipid	Mean Weight (mg)	Percent as Lipid
0/0.1	70	0.131	1.867	0.0059	0.0844	4.52
1	32	0.078	2.434	0.0062	0.1925	7.91
10	28	0.072	2.589	0.0042	0.1486	5.74
100	30	0.076	2.521	0.0047	0.1570	6.23
1000	32	0.063	1.967	0.0052	0.1637	8.33

Trial 2 (Long Term)						
Cnctrn. of Bravo	Number of Amphipods	Weight of Amphipods	Mean Weight (mg)	Weight of Lipid	Mean Weight (mg)	Percent as Lipid
0	49	0.114	2.328	0.0038	0.0767	3.30
1	45	0.118	2.627	0.0060	0.1331	5.07
10	42	0.088	2.102	0.0050	0.1195	5.69
100	42	0.100	2.375	0.0052	0.1250	5.26

Roundup, containing glyphosate marginally affected survival. Comparing the reference tanks to the highest exposure, survival decreased by only ten percent. Other indicators of effects, lipid and behaviour, were not changed over the tested concentrations. It is hard to say whether or not Roundup had a large effect on the amphipods. In the literature, LC_{50} results range from 2.3 to 43 $mg \cdot L^{-1}$, both of which are much greater than the levels that were studied in this experiment. A drop in ten percent in a population of animals in the wild is huge, but in the lab it seems somewhat inconsequential. Roundup did affect amphipods, but not as much as the other two pesticides.

Experiments were more difficult to conduct over longer (i.e., 10 days' exposures), but the increase in lipid content was confirmed for amphipods exposed to Bravo. The problems with the 10-day exposure stemmed from the fact that survival did not prove to be significant enough. Therefore, if these pesticide formulations arrive in an estuary inhabited by *Corophium volutator*, they could have adverse effects upon a population. Overall, toxicity in terms of survival is ranked highest for Lorsban, followed by Bravo and then Roundup.

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Response of the mud snails Ilyanassa obsoleta to ammonia, freshwater and pesticides-spiked seawater. J. Hellou[1,2] and K. Dunphy[1] (PO)

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The mud snail *Ilyanassa absoluta* is reported as an invasive species that is found in locations previously inhabited by a snail of similar size, with similar habitat preferences, i.e. *Littorena littorena*. The former animal is differentiated from the latter by the more elongated shape of the shell as opposed to the latter having a more rounded shell. These animals and the amphipods *Corophium volutator* are easily detected together at low tide in many beaches of the Bay of Fundy where the sediment grain size is fine (<50-250μm). They were chosen to investigate the effect of pesticide runoff on estuarine biota of the Maritimes. Our choice of invertebrates for experiments was determined by the availability of the animals, their relevance in the food chain, the ease of handling and maintaining them in the laboratory, their possible exposure in the field, as well as potential applications of the research. It is the authors' hope that behavioural research in terms of attraction or avoidance of a media containing pesticides could be used to interpret the results of benthic community studies performed in the field, where the presence and impact of pesticides is of concern. The variable abundance of different species of invertebrates, combined with chemical analyses of water and sediments along with results from laboratory experiments could, be used in a weight of evidence approach to investigate the role played by pesticide runoff in an estuary.

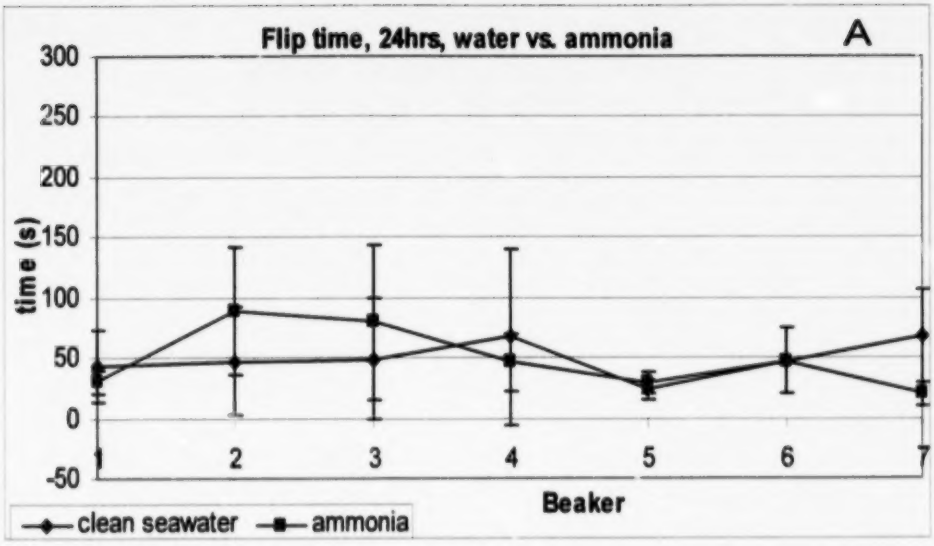
Snails, *Ilyanassa absoluta,* were exposed to atrazine, azinphos-methyl, carbofuran, chlorothalonil and endosulfan, as well as to varying levels of unionised ammonia and freshwater added to seawater, individually. Each series of experiments was conducted using one chemical at a time, except on one occasion where seawater and ammonia were combined. Behavioural endpoints covering movement and feeding ability examined if and when levels of pesticides ranging from 0.01 to 1,000 $\mu g \cdot L^{-1}$would impact animals. Overall, it was determined that, given a choice between being immersed or above contaminated water, snails were quick to escape chemical stress. Under exposure lasting 48 hrs, two additional defence responses were observed in sequence. The animals flipped on their shell with the soft tissue exposed, and then became retracted within their shell to escape exposure. These stages were easy to characterise with increasing exposure time to some of the pesticides. These three stages of response represent a simple defence mechanism to reduce exposure, balancing the need for water and air.

The snails' flip time after 24 and 48hrs (Figures 1A and B), attraction to food after 48hrs of exposure (Figure 2) and their response to probing once placed in clean seawater at the end of the experiments represented additional end points examined in our study.

Two reference beakers, a positive reference with clean seawater and a negative reference with 0.85 $mg \cdot L^{-1}$ of unionised ammonia, were prepared with each series of tested pesticides. The negative reference was prepared by adding 25mg of NH_4Cl to 1L of seawater (Alonso and Camargo, 2004). This level of unionised ammonia was reported to impact the behaviour of a freshwater snail. However, as outlined in Figure 1, the saltwater snails exposed to this concentration of ammonia did not display a change in their ability to reposition. When the standard deviations overlap between the two curves, the results are not significantly different, as confirmed with a t test. Mean and standard deviations display the results obtained for 10 snails per beaker.

Ammonia is a by-product of protein metabolism, and is released by aquatic animals. The excessive use of nutrients on agricultural fields can create a problem with increased ammonia in water runoff after a rain event. Toxicity can be affected by the pH and temperature of the water. The amount of unionised ammonia present increases when pH rises, and decreases as the pH falls. A change in pH affects the toxicity much more than a change in temperature, with pH reported to account for ~90% of the change, while temperature accounts for ~10% (Wurts, 2003).

Comparison of the data from the seawater reference and the seawater containing ammonia using a t test indicates no difference between the two sets of results. Based on this additional data, the ammonia was considered non-toxic. According to measurements performed in estuaries around Prince Edward Island, the highest detected levels of ammonia are of 2μM or 35 $\mu g \cdot L^{-1}$ (Dr. Phil Yeats, BIO, unpublished). Therefore our testing was to levels more than twenty times higher than expected.



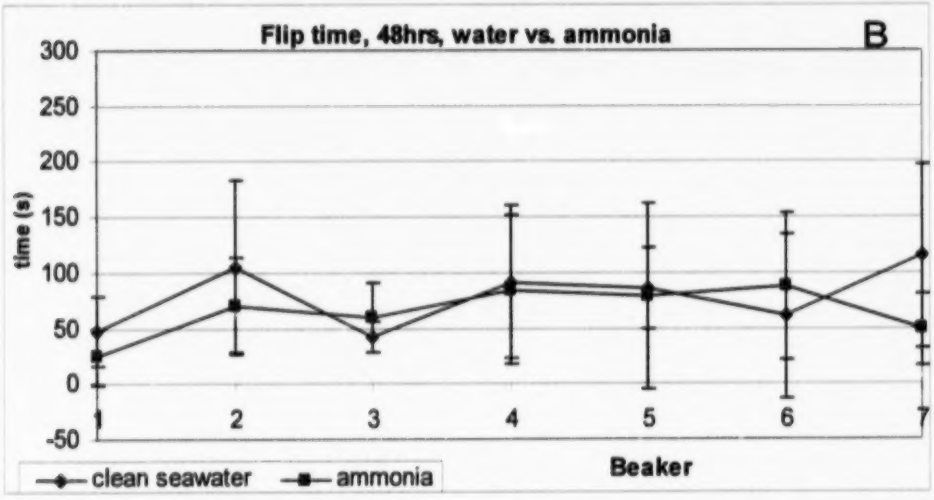


Figure 1. The flip time of the snails represents the number of seconds taken by a snail to go from lying on its shell to turning over with the soft tissue below the shell. Mean and standard deviation for the flip times presented for 24 (A) and 48hrs (B) of exposure to the ammonia solution and clean seawater conducted for seven weeks.

Exposure to increasing levels of chemicals initiated a mild or more pronounced behaviour that protected the animals, since in all cases, survival was >90% and reflected tolerance. Overall, taking into account the freshwater field results obtained by Mr. Claire Murphy, Environment Canada, Prince Edward Island, where detectable levels of pesticides (20-100 $ng \cdot L^{-1}$) were only

observed during a short period of time (<24hrs), based on our exposures to single pesticides, snails would most likely be unharmed by the discharge of the examined pesticides in an estuary.

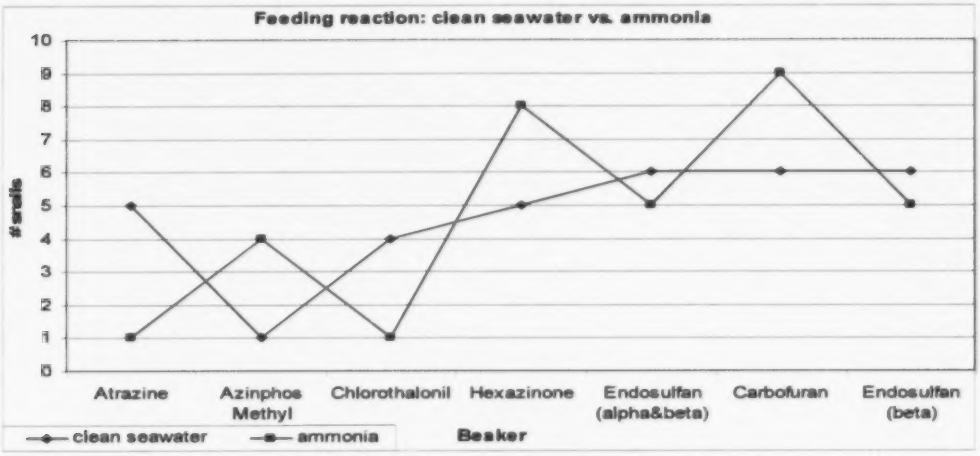


Figure 2. Number of snails that fed after 48 hrs of exposure, as observed in seven series of experiments. Results are displayed for the beakers run alongside all experiments, i.e. a seawater reference and an exposure to ammonia. The latter was expected to represent a negative response, i.e. to reflect toxicity.

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Survival and behaviour of Corophium volutator, Daphnia magna and Oncorhynchus mykiss exposed to azinphos methyl, carbofuran and endosulfan. J. Hellou[1], A. Cook[2], K. Doe[2], K. Dunphy[3], P. Jackman[2], J. Leonard and P. Walker[3] (PO)

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Pesticides sprayed on farmlands can end up in rivers and be further transported into estuaries, where they can affect aquatic organisms in freshwater as well as saltwater environments. Fish kills have been observed in rivers on Prince Edward Island and raised concern about a potential link with chemicals used to prevent diseases from affecting crops. A series of experiments was

conducted using water fleas, amphipods and rainbow trout as model organisms for potential effects in freshwater and seawater environments. Experiments used water, water with added soil, or spiked sediments to compare survival towards the presence of azinphos-methyl (AZ), carbofuran (CA) and endosulfan (EN). Behaviour was also examined in the two crustaceans. A large part of the work aimed to determine the role that sediments could play in mobilising pesticides. For that reason, it can be viewed as preliminary data that would need pursuing if the aim is to obtain more statistically significant results for future application.

The octanol-water partition coefficient reported in the literature (Kamrin 1997) ranks the pesticides' binding to sediments as EN > AZ > CA, with values of 3.66-3.83, 2.69-2.75 and 1.2-1.4, respectively. Narcotic risk, for water exposure of trout over 1, 4 and 10 hrs was highest for EN > AZ > CA (Table 1). Pesticides immobilized the water fleas at concentration several orders of magnitude below lethal levels even in the presence of particles in water. This experiment also indicated that if freshwater containing pesticides encounter moving particles for a short period of time (seconds) in a proportion of 10g per liter, the solution would impact the survival of the animals at the stated levels. Toxicity would not be reduced by the presence of sediments, thus pesticides would remain in the soluble form.

Corophium volutator exposed to pesticides spiked in sediments displayed the opposite toxicity ranking CA and AZ > EN in 48hrs experiments. These amphipod experiments were exploring the potential binding of pesticides to sediments and the effect of this media. In the present case, the pesticides would have arrived directly in the particles perhaps deposited as soil, then entrained with a disturbance into inter-tidal sediments. These experiments were only performed in duplicate, for each level of exposure. They appear to indicate that the more water-soluble pesticides and least bound to sediments, CA and AZ, are also less toxic to the sediment-dwelling animals. The LC_{50} for these pesticides is of 73, 73 and 232 $ng \cdot g^{-1}$, respectively, as calculated using linear regressions, Log-Logit (corrected for heterogeneity). LC_{20} was of 41, 41, 130 $ng \cdot g^{-1}$ for the same pesticides (Table 2).

Table 1. Comparison of dose inducing lethality relative to exposure time with rainbow trout. Concentrations are expressed in $\mu g \cdot L^{-1}$.

Pesticide	Highest concentration from 2001-2005 buffer zone study	Highest concentration from PEI fish kills	1 h LC50 trout	4 h LC50 trout	10 h LC50 trout
Azinphos methyl	15.7	261	400	71.2	30.7
Endosulphan	610.4	86	100	29.1	19
Carbofuran	354.4	39	1270	1130	800

The avoidance/preference behaviour of the animals relative to spiked/reference sediments was judged using observations performed over several years where animals exposed to reference/reference sediments were distributed over the two halves of a tank in a 40 to 60% ratio in more than 80% of the exposures (Hellou *et al.*, 2005). However, in experiments performed alongside the spiked sediments, there was only a 62% chance of detecting the animals exposed to reference sediments in two halves of a tank in a ratio of 40-60%. It is possible that our experimental setup is such that there is cross-contamination with the used pesticides, either due to their volatility or to human error. Therefore, the behavioural endpoint represents a tendency that needs to be ascertained by performing additional studies. Amphipods' avoidance of pesticide-spiked sediments relative to preference of reference sediments pointed to repellent properties for EN when spiked at level >90 $ng \cdot g^{-1}$, a potential attraction to CA at <40 $ng \cdot g^{-1}$, while AZ did not affect behaviour. Behavioural studies offer a non-lethal approach to assess environmental health and their use in risk assessments should be pursued.

Table 2. Regression for the preference of pristine sediments relative to the pesticide concentrations (1.4% TOC) in 2 days' experiments. 1. Number of data point used in the regressions. 2. Behaviour representing a preference for reference sediments; NO: not observed means no significant regressions with $p < 0.05$. All r^2 represent a level of significance better than 5%

Pesticide (n)[1]	Behaviour[2] {r^2}	LC_{50}	LC_{20}
Azinphos methyl (7)	NO	73	41
Carbofuran (8)	-0.234x + 49.423 {0.78}	73	41
Endosulfan (8)	0.129x + 48.232 {0.78}	232	130

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